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STUDIES ON LEUKOCYTOSIS *
III. HOURLY DETERMINATIONS OF THE MATURITY OF NEUTROPHILS OF NORMAL RABBITS

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In connection with studies on leukocytosis in rabbits, the question has arisen whether the maturity of polymorphonuclear neutrophilic leukocytes (amphophils) varies from hour to hour during the day. As a review of the literature shows no reported studies of this variation, results are presented of 21 such determinations made on 20 rabbits.

PROCEDURE

Total and differential leukocyte examinations were made at hourly intervals, at approximately the same hours during the same period of the day, of 20 adult normal rabbits, studied during the period from May 24, 1939 to December 30, 1940. These results and the various general procedures involved in leukocyte determinations, such as feeding, housing, selection of animals and obtaining of blood samples, have been described previously.[1] Special consideration was given to the exact duplication of these methods and to all other procedures to ensure the utmost accuracy of the results.

The method used in classifying neutrophils is based on a report by Pons and Krumbhaar,[2] the cells being arranged in four groups according to the modification of Hunt and Weiskotten,[3] as follows:

Group I. Cells with the nuclear material in one mass, round, oval, or indented not more than one-half through its width.

Group II. Cells with the nuclear material not divided into segments, but it may be lobed, spiral, looped, rosette-shaped or variously irregular.

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Group III. Cells with the nuclear material showing segmentation into two parts which are either entirely separate (as viewed in the smear), or connected by a narrow filament.

Group IV. Cells with the nuclear material showing segmentation (as in group III) into more than two parts.

The relation of this classification to other classifications of neutrophils by the appearance of the nuclear material is shown in Table I. It is seen that groups I and II and groups III and IV comprise non-filamented and filamented neutrophils respectively. While further subdivision of the group IV cells, dependent upon the number of nuclear parts (more than two), might seem advisable, in our experience such finer classification is not practical because in many neutrophils the nu-

TABLE I

Comparison of Methods of Classifying Neutrophilic Maturity by Nuclear Appearance

Method of classification	Type of cell of neutrophilic series					
	Non-filamented cells		Filamented cells			
	Myelocyte, metamyelocyte	Nonsegmented, band, staff or stab forms; "young neutrophils"	Segmented forms*			
			2 lobes	3 lobes	4 lobes	5 lobes
Arneth count[4]	I	I	II	III	IV	V
Polynuclear count (Cooke and Ponder[5])	I	I	II	III	IV	V
Method used in present study	I	II	III	IV	IV	IV

* Our criterion of segmentation, like that of Cooke and Ponder,[5] is more strict than the criterion of Arneth.[4]

clear parts are not separated sufficiently to show more than three parts, but are adjacent to or superimposed on each other.

Hunt and Weiskotten,[3] using this method in a study of the maturity of rabbit neutrophils (amphophils) liberated from a regenerating bone marrow, concluded that a "shift to the left" in the count (increase in percentage of the simpler formed nuclei—groups I and II) actually indicated a relative increase in the number of young or more immature neutrophils in the circulating blood.

During the course of various studies on leukocytosis involving neutrophilic nuclear classifications, approximately 60,000 neutrophils have been examined and grouped.

With a few exceptions, at least 100 neutrophils have been examined and classified for each determination. These exceptions in which at least 50 and usually more neutrophils were examined, occurred when blood films were very thin or smears were taken at times when the

absolute numbers of neutrophils present in the circulating blood were very low (*e.g.*, 200 to 300 per cmm.). Under such circumstances the entire surfaces of from one to three smears have been examined. As repeated comparisons of the results of classifications of 50 successive neutrophils on the same smear have shown a close correlation, the results when at least 50 neutrophils have been examined were felt to be sufficiently accurate to be acceptable. Cooke and Ponder [5] stated that the probable error of the mean was approximately ± 0.05 when 100 neutrophils were counted, and ± 0.07 when 50 cells were counted.

Neutrophils in which the nuclear masses were not stained well or were not clearly outlined, were discarded. The number of discarded neutrophils was less than 5 per cent in any experiment. Care was taken to distinguish between poor staining in general and the irregular and atypical staining which sometimes is evident in non-filamented neutrophils.

The thinness of the narrow filament between nuclear parts in neutrophils of groups III and IV was usually quite characteristic. We have used Cooke's [5] criterion on nuclear lobulation: "If there is any band of nuclear material except a chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purpose of the count, be said to be divided." If any nuclear detail caused the question to arise in which of two groups a cell should be classified, the cell arbitrarily was placed in the group "to the right." Such a procedure was occasionally, but not frequently, necessary.

The weighted mean, as described by Cooke and Ponder,[5] was used to express the maturity of 100 neutrophils by a single figure. To determine the weighted mean, the number of cells in each group is multiplied by the number of the group, all results are added and this sum is divided by the total number of cells counted. Thus the count:

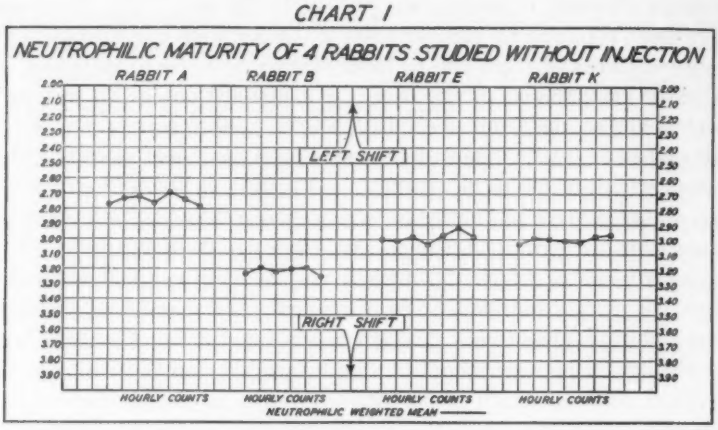
I	II	III	IV
0	31	34	35

gives a weighted mean of 3.04, that is, the average age of these 100 neutrophils is group 3.04. As Cooke and Ponder have pointed out, the weighted mean is a very sensitive index of the nuclear classification, for a difference of one point in the second decimal place means that one neutrophil has been moved to the group either below or above its original group.

Results

Neutrophilic weighted means of successive hourly counts in 21 experiments on 20 rabbits are shown in Table II, and typical hourly curves for 4 of these animals are illustrated in Chart 1. Study of Table II

CHART 1



shows that each rabbit on its particular day of counting seemed to have a rather characteristic weighted mean, some more "to the left" than others. Regardless of the value of this weighted mean, there was no apparent tendency for the mean to shift to the "left" or "right" during successive hourly counts of this period of study. Chart 1 illustrates the constancy with which the initial average neutrophilic maturity was maintained during the period of counting. Charts of the remaining 17 rabbits showed similar curves.

TABLE II

Neutrophilic Weighted Means of Successive Hourly Counts on 21 Rabbits

Rabbit letter	Weighted means of successive hourly counts							Average weighted mean
	1	2	3	4	5	6	7	
A	2.77	2.73	2.72	2.76	2.69	2.74	2.78	2.74
B	3.23	3.19	3.22	3.20	3.19	3.25	....	3.21
B	3.30	3.39	3.44	3.40	3.34	3.29	3.31	3.35
C	3.14	3.15	3.09	3.08	3.08	2.99	....	3.09
D	3.15	3.14	3.08	3.08	3.07	3.04	....	3.09
E	3.00	3.01	2.98	3.03	2.97	2.92	2.98	2.98
F	3.10	3.01	3.10	3.08	3.05	3.04	2.98	3.05
G	2.93	2.93	2.96	2.96	3.02	2.93	2.97	2.96
H	3.14	3.09	3.09	3.08	3.03	3.03	2.99	3.06
I	2.96	3.00	2.95	2.99	2.96	3.00	2.98	2.97
J	3.02	3.07	3.06	3.00	2.98	2.98	....	3.02
K	3.03	2.99	3.00	3.01	3.02	2.98	2.97	3.00
L	2.75	2.82	2.77	2.76	2.77	2.79	2.84	2.79
M	2.96	2.97	3.02	3.01	2.96	2.91	2.99	2.97
N	3.30	3.20	3.16	3.21	3.12	3.21	3.18	3.20
S	3.21	3.16	3.28	3.11	3.13	3.03	3.17	3.15
T	3.23	3.26	3.18	3.00	3.12	3.04	3.10	3.13
W	2.83	2.95	2.85	2.93	2.89	2.92	2.90	2.90
X	3.16	3.04	3.11	3.08	3.04	3.16	3.05	3.09
Y	3.02	2.98	3.06	2.97	3.03	3.03	3.02	3.02
Z	2.94	2.94	2.88	2.88	2.92	2.94	2.93	2.92

There were no evident variations dependent upon seasonal changes or the sex of the experimental animals.

The percentages of non-filamented neutrophils (groups I and II) ranged from 11 to 48, with a mean of 28.5, median of 29 and mode of 29. Those animals having the lowest and highest non-filamented neutrophil percentages also maintained the same general percentages throughout the successive hours of counting. Only two group I neutrophils were observed in classifying approximately 14,000 neutrophils.

In Table III are listed single neutrophilic maturity counts of rabbit B at approximately the same time of day (9:45 a.m.) on different days during a 23-month period. The data presented in this table are typical of those for the other rabbits in this series. It has been our experience that while the level of the neutrophilic weighted mean in a rabbit may

TABLE III

Neutrophilic Maturity Classifications of Rabbit B at Approximately the Same Time of Day on Different Days

Date	Neutrophilic classification				
	Group I	Group II	Group III	Group IV	Weighted mean
1-17-39	0	15	45	40	3.25
5-24-39	0	19	39	42	3.23
12- 5-39	0	15	40	45	3.30
12-29-39	0	14	34	52	3.38
3-28-40	0	31	38	31	3.00
7-17-40	0	19	40	41	3.22
9- 5-40	0	16	36	48	3.32
12-14-40	0	22	36	42	3.20

vary slightly over a long period of time, the relative constancy of the mean, established for any particular daily period of study, is maintained during this period of study.

DISCUSSION

The striking daily constancy of the weighted mean for each animal at its particular level seems to indicate that, as in a study of the hourly differential leukocyte picture during the same period, general trends should be established for each rabbit. After this, we should have every reason to expect very little shift to the "left" or "right" during the succeeding 6 hours. The maximum variation of the weighted mean in any experiment was 0.23. Cooke and Ponder,[5] classifying 1000 neutrophils of one blood film, observed a maximum variation of their weighted mean of 0.28.

Previous studies [1] have shown that there is a tendency for the absolute numbers of neutrophils in rabbits to increase slightly to moderately

during the afternoon hours. If we consider any neutrophil count, quantitative or qualitative, to represent the balance between constant production and constant destruction of neutrophils, the lack of "left shift" accompanying the frequent afternoon neutrophilic increases would seem to indicate that the ratio of appearance of younger neutrophils in the blood to the disappearance of older neutrophils from the blood was balanced or constant.

The scarcity of group I cells (0.014 per cent) seems to indicate that under normal conditions in these rabbits, with very rare exceptions, cells of the neutrophilic series were not discharged from the bone marrow into the circulating blood unless they were of a certain maturity, *i.e.*, more mature than the metamyelocytic stage (group I).

Summary

Variations of maturity of polymorphonuclear neutrophilic leukocytes (amphophils), expressed as a single figure, the weighted mean, are presented for 20 apparently normal rabbits, studied at successive hourly intervals during a 6-hour daily period.

Each rabbit seemed to have a characteristic degree of neutrophilic maturity on the day on which counting occurred. Regardless of this degree, neutrophilic maturity was relatively constant during the successive hours of counting.

In these rabbits, with very rare exception, only cells of the neutrophilic series which were more mature than metamyelocytes (group I) were observed in the circulating blood.

REFERENCES

1. Reifenstein, G. H.; Ferguson, J. H., and Weiskotten, H. G. Studies on leukocytosis. I. Hourly leukocyte variations of normal rabbits. *Am. J. Path.*, 1941, **17**, 219–231.
2. Pons, Carlos, and Krumbhaar, E. B. Studies in blood cell morphology and function. No. III—Extreme neutrophilic leukocytosis with a note on a simplified Arneth count. *J. Lab. & Clin. Med.*, 1924–25, **10**, 123–126.
3. Hunt, Emily, and Weiskotten, H. G. The value of the Arneth count in determining the age of neutrophile (amphophile) leucocytes (rabbit). The action of benzol. VIII. *Am. J. Path.*, 1930, **6**, 175–181.
4. Arneth, Joseph. Die neutrophilen weissen Blutkörperchen bei Infektionskrankheiten. G. Fischer, Jena, 1904.
5. Cooke, W. E., and Ponder, Eric. The Polynuclear Count. The Nucleus of the Neutrophil Polymorphonuclear Leucocyte in Health and Disease, with Some Observations on the Macropolycyte. Charles Griffin & Company, Limited, London, 1927.

STUDIES ON LEUKOCYTOSIS *

IV. The Neutrophilic Maturity Following Intravenous Injection of Supernatant Fluid from a Sterile Exudate (Rabbit)

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Previous studies [1] have indicated that an absolute neutrophilic leukocytosis regularly occurs in rabbits several hours following the intravenous injection of supernatant fluid from a sterile, sodium chloride-induced exudate from the peritoneal cavity of a rabbit. Apparently some substance or substances, not sodium chloride, present in these exudates, produced these neutrophilic leukocytoses.

While it is possible that a neutrophilic leukocytosis may result from an altered distribution of neutrophils in various organs, it is generally agreed that an increase in the number of more immature neutrophils in the circulation (so-called "left shift") can result only from release of younger neutrophils from the bone marrow. It would seem that studies of the neutrophilic maturity following intravenous injection of supernatant fluid from a sterile exudate might give more information on this point.

In the present study, hourly variations in neutrophilic maturity and total and differential leukocyte counts were determined for 11 rabbits, each studied at the same hourly intervals during the same daily period, without injection and before and after single and repeated injections of 5 cc. of supernatant fluid from a sterile rabbit exudate. Four of these rabbits received in addition single injections of 25 cc. of supernatant fluid. Similar studies were made on 5 of these rabbits following single or repeated control injections of 5 cc. of sterile 0.9 to 0.78 per cent sodium chloride solution.

Procedure

The procedures involved in the selection, feeding and housing of these rabbits; the determinations of total and differential leukocyte counts and variations in neutrophilic maturity, and the preparation, characteristics and injection of the sterile rabbit exudates have been described

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An abstract of some of the results of this investigation was read by title at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941, and was read at the organization meeting of the American Federation for Clinical Research, May 5, 1941, and at the annual meeting of the New York State Association of Public Health Laboratories, May 19, 1941.

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in detail elsewhere.[1-3] These procedures may be summarized briefly as follows: exudates were produced by the intraperitoneal injection into rabbits of 300 cc. of sterile 0.9 per cent sodium chloride solution. The fluid exudate was withdrawn 5¾ to 15½ hours later, centrifugated, and either 5 or 25 cc. of the supernatant fluid fraction immediately injected intravenously into the rabbit to be studied. In the case of repeated injections, the supernatant fluid was kept at room temperature during the period of injections.

The sodium chloride concentrations of some of these supernatant fluid fractions had previously been determined.[1] Sterile sodium chloride solutions of equivalent concentrations were injected intravenously in equivalent amounts into five of these animals.

The repeated injections consisted of four 5 cc. injections at hourly intervals beginning at 10:15 a.m. The experiments involving repeated supernatant fluid injections preceded the experiments involving single supernatant fluid injections in four animals. In the other seven animals, repeated injection experiments followed single injection experiments. All repeated control injections of saline solutions were given after supernatant fluid injections had previously been made into the same rabbits. The interval between any two experiments ranged from 14 to 140 days.

Total and differential leukocyte counts and determinations of neutrophilic maturity of these 11 rabbits were made under the varying experimental conditions at hourly intervals at the same times of day during the same 6-hour daily period. Subsequent leukocyte studies were also made at intervals varying up to 101½ hours after an injection. The weighted mean [3] was used to express the degree of neutrophilic maturity of each determination as a single figure. Every attempt was made to have all factors of these experiments constant except the amounts and types of material injected.

Results

Results are presented in Tables I to IV and Charts 1 to 4.

In a series of 25 experiments, 11 rabbits received both single and repeated injections of 5 cc. of supernatant fluid fractions of sterile rabbit exudates. A neutrophilic leukocytosis, comparable in extent and time of occurrence to those previously described,[1] occurred in every experiment. In each experiment this leukocytosis was accompanied by a decrease of the neutrophilic weighted mean, indicating a "shift to the left" or appearance of a greater proportion of younger or more immature cells in the circulating neutrophil population. The hourly neutrophilic weighted means in these 25 experiments are shown in Table I.

There was no evident correlation between these leukocytoses with

"left shifts" and the duration of the exudate or the particular animals in which the exudates were produced. When a supernatant fluid fraction of the same exudate was injected into two rabbits, there was no evident correlation between the neutrophilic changes in the two rabbits.

As a decrease of the weighted mean may result from a relative de-

TABLE I

Hourly Neutrophilic Weighted Means in 25 Experiments on 11 Rabbits Receiving Intravenous Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Type of injection	Hourly neutrophilic weighted means							Time after injection at which count no. 2 was taken (hours)*
		1	2	3	4	5	6	7	
B	Single	3.38	3.10	2.89	2.94	2.89	2.86	3.13	1
B	Single	3.00	2.78	2.72	2.59	2.49	2.56	2.85	1
B	Repeated	3.32	3.00	2.56	2.31	2.32	2.29	2.18	½
F	Single	3.08	3.07	2.58	2.56	2.47	2.53	2.83	½
F	Single	2.99	2.71	2.59	2.55	2.69	2.77	2.88	1
F	Repeated	†	3.12	2.84	2.68	2.89	2.91	2.68	½
I	Single	2.85	2.60	2.62	2.55	2.57	2.78	2.80	1
I	Single	2.76	2.58	2.49	2.61	2.59	2.66	2.88	5/6
I	Repeated	3.16	3.18	2.88	3.04	3.03	3.05	2.94	½
M	Single	2.84	2.55	2.51	2.46	2.48	2.45	2.85	1
M	Repeated	3.02	2.90	2.65	2.45	2.30	2.26	2.15	½
N	Single	3.14	2.82	2.72	2.65	2.71	2.84	2.92	1
N	Repeated	3.18	2.87	2.68	2.50	2.36	2.30	2.23	½
S	Single	3.02	3.02	2.73	2.64	2.71	2.86	2.94	½
S	Repeated	3.06	....	2.30	2.43	2.27	2.10	2.19	‡
T	Single	3.12	2.90	2.81	2.71	2.77	2.79	2.89	½
T	Repeated	2.96	....	2.50	2.42	2.42	2.30	2.33	‡
W	Single	3.07	2.84	2.72	2.64	2.82	2.86	2.94	½
W	Repeated	2.90	2.70	2.38	2.22	2.28	2.20	2.26	½
X	Single	2.92	2.85	2.82	2.69	2.83	2.93	2.89	½
X	Repeated	3.05	2.76	2.38	2.28	2.28	2.34	2.26	½
Y	Single	2.97	2.80	2.78	2.67	2.73	2.74	2.84	½
Y	Repeated	3.04	2.82	2.44	2.37	2.27	2.36	2.23	½
Z	Single	2.83	2.80	2.63	2.52	2.61	2.60	2.70	½
Z	Repeated	3.00	2.80	2.43	2.14	2.23	2.27	2.26	½

* Hours after first injection, in the case of repeated injections.
† No determination (unsatisfactory stain).
‡ No. 3 count 1¾ hours; no. 4 count 2½ hours.

crease in the absolute numbers of older neutrophils present in the circulation, the absolute number of neutrophils in groups I, II, III and IV were calculated in all experiments. Table II shows the results of neutrophil studies of rabbit I without injection and before and after an intravenous injection of 5 cc. of supernatant fluid. This table shows that the decreased neutrophilic maturity was due to an increased number of more immature neutrophils in the circulation. The absolute numbers of more mature neutrophils seemed to remain relatively constant during the period of counting. Similar results were obtained in the other ten animals.

As a control procedure, leukocyte studies were made on five of these rabbits after single or repeated injections of 5 cc. of sterile sodium chloride solution. In every experiment the absolute numbers of neutrophils at successive hours varied no more than when studied without injection during the same daily period, and the neutrophilic maturity

TABLE II

Absolute Numbers of Neutrophils per Cubic Millimeter in Each Group of Maturity (Rabbit I)

	Time	Absolute numbers of neutrophils per cmm.	Classification of neutrophilic maturity			
			Group I	Group II	Group III	Group IV
Experiment 39.43—Dec. 11, 1939—without injection	9:45 a.m.	1,342	0	416	564	362
	10:45 a.m.	1,166	0	373	420	373
	11:45 a.m.	1,414	0	481	523	410
	12:45 p.m.	1,392	0	418	570	404
	1:45 p.m.	1,620	0	518	648	454
	2:45 p.m.	1,890	0	548	794	548
	3:45 p.m.	1,575	0	457	693	425
Experiment 40.1—Jan. 8, 1940—5 cc. supernatant fluid injected intravenously at 9:45 a.m.	9:30 a.m.	1,008	0	413	333	262
	10:45 a.m.	2,160	0	1,166	692	302
	11:45 a.m.	5,264	0	2,527	2,211	526
	12:45 p.m.	4,046	0	2,266	1,335	445
	1:45 p.m.	3,780	0	1,928	1,550	302
	2:45 p.m.	3,392	0	1,357	1,424	611
	3:45 p.m.	2,508	0	1,003	1,003	502
	12½ hours*	2,145	0	858	772	515
	24¾ hours*	1,134	0	363	567	204

* After injection.

remained relatively constant. The maximum decrease in the weighted mean in any control experiment was 0.09, which was well within the limits of normal variation.

Comparison of the numbers and maturity of the neutrophils of three animals under the varying experimental conditions is possible from Charts 1, 2 and 3 (rabbits B, N and F respectively). These charts are typical of all experiments on all 11 rabbits. The dates of each experiment indicate the order in which the various procedures were performed. Chart 1 shows that the hourly neutrophilic maturity of rabbit B was relatively constant without injection and following repeated injections of 5 cc. of sterile sodium chloride solution. Neutrophilic leukocytoses and "left shifts" occurred after both experiments involving supernatant fluid injection. The "left shift" following a single 5 cc. injection reached its maximum approximately 2 hours after injection. After repeated 5 cc. injections the neutrophilic "left shift" was greater and more progressive and the neutrophilic leukocytosis somewhat more progressive. Chart 2 presents similar results of identical experiments on

rabbit N. Chart 3 (rabbit F) shows that after repeated injections of supernatant fluid, a maximum neutrophilic leukocytosis occurred, while the degree of "left shift" was approximately the same as that observed following a single injection of 5 cc. of supernatant fluid.

CHART 1 RABBIT B

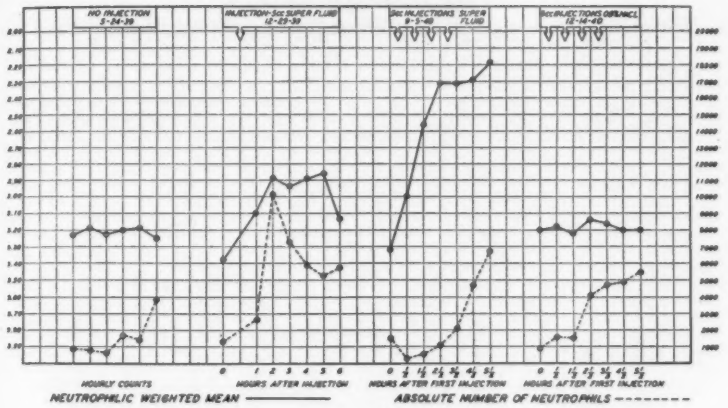


CHART 2 RABBIT N

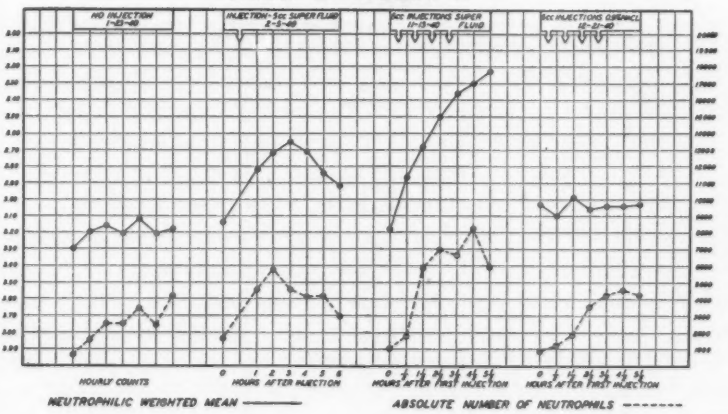


Table III shows the maximum decrease of the neutrophilic weighted mean ("left shift") which occurred in each rabbit following single and repeated injections of 5 cc. of supernatant fluid. Following single injections, the maximum "left shifts" occurred approximately 2 to 4 hours after injection. In all animals except rabbits F and I, the "left shifts" were considerably greater and tended to progress, rather than to

peak, during the consecutive hours of observation after repeated injections. However, animals F (Chart 3) and I, although having "left shifts" which were approximately the same after both single and repeated injections, showed their highest neutrophilic leukocytoses after repeated injections. The neutrophilic maturity returned to a normal

CHART 3 RABBIT F

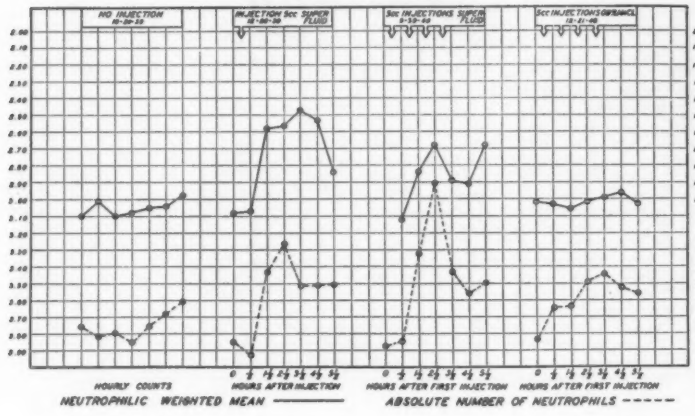


TABLE III

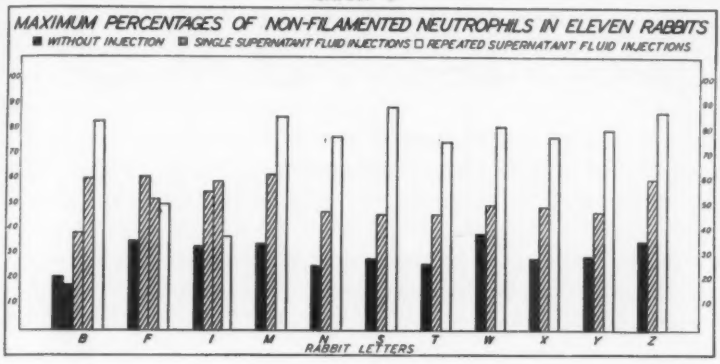
Maximum Decreases of Neutrophilic Weighted Means ("Left Shifts") in 11 Rabbits Receiving Single and Repeated Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Single injection	Repeated injections
B	0.42 0.51	1.14
F	0.61 0.44	0.44
I	0.30 0.27	0.28
M	0.39	0.87
N	0.49	0.95
S	0.38	0.96
T	0.41	0.67
W	0.43	0.70
X	0.23	0.80
Y	0.30	0.81
Z	0.31	0.86
Average	0.39	0.76

level from 4½ to 24¼ hours (average 9 hours) after a single 5 cc. injection, and from 7½ to 72 hours (average 45½ hours) after the first of four repeated 5 cc. injections.

The maximum percentages of non-filamented neutrophils (groups I and II) observed in studies of these 11 rabbits without injection, and before and after single and repeated injections of 5 cc. of supernatant fluid are shown in Chart 4. It is evident that in 9 of these 11 rabbits,

CHART 4



approximately 70 to 90 per cent of the circulating neutrophils were non-filamented at the maximum of the "left shift" following repeated injections. The maximum increases in percentages of non-filamented neutrophils observed in these 11 rabbits, studied under these conditions, are presented in Table IV. Despite the large increases in percentages of non-filamented neutrophils after supernatant fluid injection, only 12 group I neutrophils (metamyelocytes) were observed during this study of approximately 41,000 neutrophils. Thus the group II cells almost exclusively constituted the non-filamented neutrophilic population in these experiments.

The neutrophilic changes following a single intravenous injection of 25 cc. of supernatant fluid were studied in four additional experiments on four of these rabbits. The neutrophilic leukocytosis which occurred in each was similar in extent and time of occurrence to the leukocytoses after single or repeated 5 cc. injections. The neutrophilic "left shifts" accompanying these leukocytoses in general were intermediate in extent between the "left shifts" following single 5 cc. and repeated 5 cc. injections. The maximum decreases of the weighted means in these four experiments ranged from 0.48 to 0.74, averaging 0.63.

Discussion

Hourly determinations of the neutrophilic maturity of rabbits studied without injection have shown a striking tendency for the index of maturity to remain constant.[3] This constancy may be contrasted with the neutrophilic "left shifts" which occurred in these same rabbits following intravenous injections of supernatant fluid fractions of sterile exudates.

Table IV

Maximum Increases in Percentages of Non-filamented Neutrophils in 11 Rabbits Receiving Single and Repeated Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Without injection	Single 5 cc. injection	Repeated 5 cc. injections
B	2 3	25 30	68
F	4	33 24	27
I	3	15 20	14
M	1	29	60
N	6	25	60
S	0	27	66
T	11	27	48
W	0	21	46
X	3	16	55
Y	4	24	53
Z	4	24	57
Average	3.5	24	50

Examination of Table II shows that these "left shifts" resulted from increases in the numbers of more immature neutrophils in the circulating blood. It seems more probable that this neutrophilic immaturity was effected by a release of younger neutrophils from the bone marrow, rather than an alteration of neutrophilic distribution in organs other than the bone marrow.

It would seem that a marked release of these more immature neutrophils must have occurred when marked neutrophilic "left shifts" occurred, such as after repeated injections of supernatant fluid. Despite the repeated observation that from 70 to 90 per cent of the neutrophils were more immature (non-filamented) at the maximum of the "left shift," group I neutrophils (metamyelocytes) very rarely were observed

to be present in the circulating blood. This scarcity of group I cells also has been observed in studies of these rabbits without injection.[3] Cells of the neutrophilic series more immature than metamyelocytes never were observed in the circulating blood in these experiments.

In general, the results of these experiments indicate that rabbits receiving both single and repeated injections of 5 cc. of supernatant fluid fractions, regardless of the order of the injections, had neutrophilic "left shifts" which were greater in extent and more progressive following the repeated injections. Stated in another way, following repeated injections there was a greater number of more immature neutrophils in the circulation for a longer period of time. These observations suggest that a "summation" of "shifts to the left" of the neutrophilic maturity occurred after repeated injections.

As far as these experiments are concerned, there seems to be no justification for assuming that these neutrophilic changes occurred because of conditioning of the rabbits by previous injection of supernatant fluid.

Neutrophilic "left shifts" have been reported to have followed various procedures, including the administration of bacteria,[4, 5] various split proteins,[6, 7] thyroid extract,[8] and x-ray[9] and ultraviolet irradiation.[10] Ponder and Macleod[11] presented the results of one experiment in which an approximately comparable neutrophilic "left shift" followed the intraperitoneal injection into a rabbit of 10 cc. of supernatant fluid from a peritoneal exudate induced in a rabbit by a saline solution. Menkin[12] reported that neutrophilic immaturity followed the intracardiac injection into dogs of the pleural exudate induced in a dog by turpentine. The relation of these "left shifts" reported in the literature to those observed in the present experiments is uncertain.

As has been indicated previously,[1] the chemical and physical nature of the substance or substances in these supernatant fluid fractions of sterile exudates which produced these neutrophilic changes is unknown. Routine bacteriological checking has yielded negative results. Leukocyte studies following single and repeated intravenous injections of 5 cc. portions of sterile sodium chloride solutions into these same animals would seem to eliminate the possibility that sodium chloride *per se* produced these neutrophilic changes.

Summary

Supernatant fluid fractions of centrifugated 5¾- to 15½-hour sterile peritoneal exudates from rabbits were obtained as described previously.[1] Twenty-five experiments were performed, in which 11 rabbits each received single and repeated intravenous injections of 5 cc. of these supernatant fluid fractions. In each experiment there occurred a

neutrophilic leukocytosis and a neutrophilic "left shift." These "left shifts" were due to increased numbers of more immature neutrophils in the circulating blood.

The "left shifts" following single injections tended to reach a peak 2 to 4 hours after injection. Repeated injections were followed by considerably greater and more progressive "left shifts" in 9 of the 11 rabbits. In the other 2, repeated injections were followed by considerably greater neutrophilic leukocytoses. Neutrophilic leukocytoses were approximately equal in all other experiments. The "left shifts" after single injections of 25 cc. of supernatant fluid in four additional experiments on 4 of these rabbits, in general, were intermediate in extent between the "left shifts" following single 5 cc. and repeated 5 cc. injections.

Group I neutrophils (metamyelocytes) very rarely were observed in the circulating blood, even when marked "left shifts" (70 to 90 per cent non-filamented neutrophils) were present.

Control experiments, in which 5 of these 11 rabbits received single or repeated injections of 5 cc. of sterile sodium chloride solutions of equivalent concentrations, showed no neutrophilic leukocytoses or "left shifts."

Conclusions

Some substance or substances, not sodium chloride, present in supernatant fluid fractions of sterile peritoneal exudates from rabbits, produced neutrophilic "left shifts" when injected intravenously into other rabbits. Following repeated injections of supernatant fluid, there was an apparent "summation" of these "left shifts."

These neutrophilic "left shifts" were effected by release of more immature neutrophils in greater numbers, probably from the bone marrow. In these experiments, metamyelocytes (group I neutrophils) were observed very rarely, and no cells more immature than metamyelocytes were found in the circulating blood.

REFERENCES

1. Reifenstein, G. H.; Ferguson, J. H., and Weiskotten, H. G. Studies on leukocytosis. II. Neutrophilic leukocytosis following intravenous injection of supernatant fluid from a sterile exudate (rabbit). *Am. J. Path.*, 1941, **17**, 233–250.
2. Reifenstein, G. H.; Ferguson, J. H., and Weiskotten, H. G. Studies on leukocytosis. I. Hourly leukocyte variations of normal rabbits. *Am. J. Path.*, 1941, **17**, 219–231.
3. Reifenstein, G. H., and Hilfinger, M. F., Jr. Studies on leukocytosis. III. Hourly determinations of the maturity of neutrophils of normal rabbits. *Am. J. Path.*, 1942, **18**, 363–368.

4. Cooke, W. E. The Arneth count in pulmonary tuberculosis. *Brit. J. Tuberc.*, 1914, **8**, 211–217.
5. Ponder, Eric, and Flint, K. N. Studies on the Arneth count.—VII. Experimental tuberculosis in the rabbit. *Quart. J. Exper. Physiol.*, 1927–28, **18**, 45–54.
6. Ponder, Eric, and Flint, K. N. Studies on the Arneth count.—VI. The effect of various drugs and extracts. *Quart. J. Exper. Physiol.*, 1926–27, **16**, 393–398.
7. Danzer, M. Studies on the Arneth count.—XV. The effect of tissue injury. *Quart. J. Exper. Physiol.*, 1930, **20**, 141–147.
8. Ponder, Eric. Studies on the Arneth count.—IV. The deflection of the count by thyroid injections. *Quart. J. Exper. Physiol.*, 1926–27, **16**, 227–239.
9. Kennedy, W. P., and Grover, C. A. Studies on the Arneth count.—VIII. The deflection of the count by X rays. *Quart. J. Exper. Physiol.*, 1927–28, **18**, 79–87.
10. Kennedy, W. P., and Thompson, W. A. R. Studies on the Arneth count.—X. The deflection of the count by ultra-violet rays. *Quart. J. Exper. Physiol.*, 1927–28, **18**, 263–266.
11. Ponder, Eric, and Macleod, John. White cell morphology in rabbits with induced peritoneal exudates. *J. Exper. Med.*, 1938, **67**, 839–846.
12. Menkin, Valy. Studies on inflammation. XVIII. On the mechanism of leukocytosis with inflammation. *Am. J. Path.*, 1940, **16**, 13–32.

MALIGNANT LYMPHOMA *

A Clinico-Pathologic Survey of 618 Cases

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Many generic terms have been utilized to designate those maladies of the lymphatic system which are characterized clinically by progressive tumor-like enlargement of lymphoid tissue with eventual fatality, and histologically by multiplication of one or more of the elements normally present in lymph nodes to the point of destruction of the nodal architecture. Of these, "malignant lymphoma" seems to have won most general usage in this country, at least, and has the advantage of being noncommittal as to pathogenesis.

Numerous attempts to subdivide this group of diseases have been complicated by an extraordinary confusion of terminology and controversial theses. In recognition of the complexity of the problem, The American Association of Pathologists and Bacteriologists established a "Registry of Lymphatic Tumors." Although no official classification has been promulgated, the publication in 1934 [1] by the registrar at that time, George R. Callender, of a review of the Registry material tacitly set the seal of the Registry's approval upon the schematization and terminology employed. This represented in fact an attempted fusion of three classifications: (1) cytologic, based upon morphologic recognition of component proliferating cells; (2) gross anatomical, depending upon the distribution of the process throughout the organs of the body; and (3) clinical, contingent upon physical signs and hematologic manifestations. In the intervening years no revision or amplification has appeared.

In the face of such authority it is with no little hesitation that we have the temerity to offer a somewhat different classification. An honest attempt over a period of years to apply the Registry terminology to our material has convinced the staff of this laboratory that it is in many respects impractical for routine use. Any considerable experience with malignant lymphoma makes it apparent that there is no necessary correlation between cytology and distribution. Any type of cell may be associated with a localized process, with a generalized disease involving multiple organs without blood manifestations, and, in all probability, with the presence of significant numbers of the type cell in the circulating blood—leukemia, in fact. It must be conceded, however, that

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many authorities will fail to recognize such relations in some of the subtypes, notably Hodgkin's disease, though Warthin [2] has recorded well documented instances. Nothing in the histologic character of an individual lesion will permit a reliable forecast as to the distribution of the process in other parts of the body. Moreover, determination of the extent and character of organ involvement is beyond the scope of ordinary clinical examination in many instances and must frequently await postmortem study, thus establishing a significant limitation, to say the least, to its clinical utility. Furthermore, another fallacy is introduced by dependence upon the results of necropsy. This shows only a single stage of the process, ordinarily, it is true, the terminal one, but not necessarily, since mechanical and surgical accidents or infection may have contributed to a lethal outcome at a comparatively early period in the evolution of the disease. More specifically, in regard to invasion of the blood stream, no constant histologic substrate permits one to predict whether or not the blood picture will prove "leukemic." Though diffuse bone-marrow involvement will be found in the great majority of leukemic patients, the marrow may be normal.[3,4] Conversely, diffuse marrow change is not infrequently found with normal blood pictures.[5,6] Still less reliable are the "leukemic" types of organ infiltration without tissue destruction such as are commonly seen in the liver, spleen and kidneys. They are frequently absent in leukemia and may be seen in typical form without leukemia. Moreover, continued observation of patients over a period of years shows significant variation in the blood picture, leukemic pictures alternating with non-leukemic ones, often quite irrespective of roentgen therapy. This feature will be dealt with in more detail in the body of this publication. Classification based upon distribution has therefore only a limited value; temporally limited because it is valid only for a given stage of the disease and must be altered from time to time in the same individual; practically limited in many instances by the impossibility of accurately determining distribution during life.

The alternative form of classification, cytologic, depends for its validity and applicability upon: (1) the constancy over months and years of observation of the reacting cell types, and (2) the possibility of histologic distinction in routine biopsy material. The first point, which is fundamental, will be made a subject for further analysis later in this paper. It is sufficient to state at this point that great constancy does in fact prevail and though variations occur they are practically limited to degree of differentiation without significant shifts between one major group and another. In respect to the second point, the applicability to routine biopsies, distinction between the well differen-

tiated lesions is simple; with less differentiation it becomes somewhat more difficult but is still feasible, and in a small number (6 per cent) of poorly differentiated lesions it becomes exceedingly difficult.

To those who may share our beliefs and regard all members of this group of diseases as neoplastic entities, the proposed classification has the advantage of conformity with the accepted usages of oncology. Classification of neoplastic disease is fundamentally cytologic, resting primarily on identification of the type cell with secondary qualification dependent on degree of differentiation rather than upon the distribution of metastasis. If such classification has more than academic value in the field of malignant lymphoma, it should show a reasonable degree of correlation with the clinical course of the disease. Such correlation is, we believe, demonstrated in Part II of this study.

The material included in this survey consists of all examples of malignant lymphoma passing through the Pathology Laboratory of the Massachusetts General Hospital in the 20-year period from January, 1917, to December, 1936, from which satisfactory histologic sections were available for personal study. This comprised 135 autopsies and 580 biopsies obtained from a total number of 618 patients. The results of a histologic survey constitute the first part of this paper. Clinical data from 545 of these have been analyzed and form the basis for Part II (Text-Fig. 1).

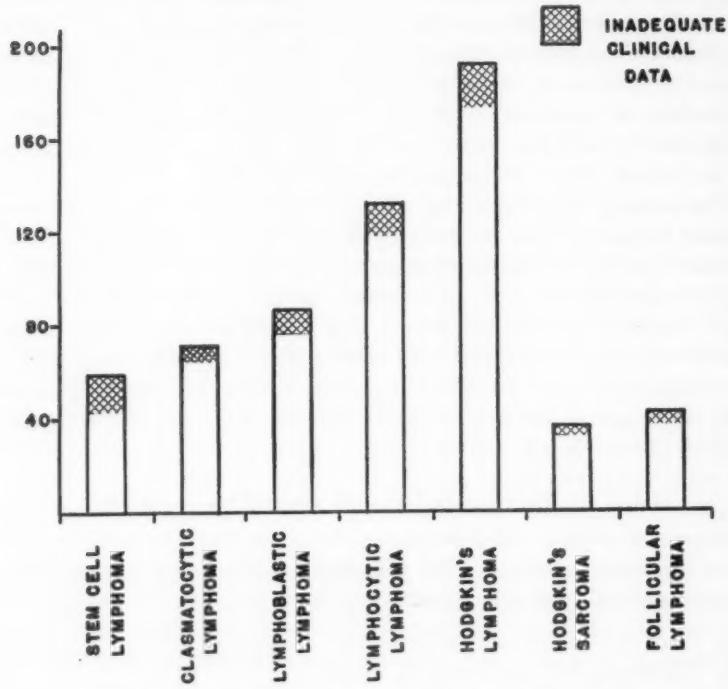
Part I. Histologic Studies and Classification

It proved easily possible to divide the great majority of the specimens into seven categories. The nomenclature adopted and the number of cases of each type were as follows:

1. Stem cell lymphoma, 56 cases } "Reticulum cell sarcoma"
2. Clasmatocytic lymphoma, 71 cases }
3. Lymphoblastic lymphoma, 85 cases
4. Lymphocytic lymphoma, 135 cases
5. Hodgkin's lymphoma, 193 cases
6. Hodgkin's sarcoma, 36 cases
7. Follicular lymphoma, 42 cases

A distinct division can be made between the first four types and the last three. The histologic pattern of the former is comparatively simple whereas that of the latter is relatively complex. Except in the cytologic peculiarities of the type cell the general structure of members of the first four categories is so similar that many features can best be described commonly for the entire group. Common to all is the tendency of proliferating cells to encroach upon, obscure and finally replace the

normal nodal architecture, reconstructing the stromal framework more or less completely in the process. Tendencies to invade marginal and medullary sinuses, to migrate through the capsules and invade perinodal tissues are representative of all four and are found irrespective of the degree of localization or generalization of the disease. In nodes showing an early stage of involvement it is frequently possible to ob-



TEXT-FIGURE 1. Frequency of the subgroups by number of cases.

serve invading strands of tumor cells projecting from an established focus into the residual normal tissue of the node. Under such conditions the reticulum fibrils of the original tissue appear to be pushed aside rather than disrupted by the invading cells, resulting in a stromal condensation which occasionally simulates encapsulation. This, however, is but a transient phase and eventually, with complete invasion, all evidence of this condensed reticulum disappears and a delicate fibrillar network completely replaces the preëxisting stroma (Fig. 20).

A problem in histologic interpretation common to all members of the lymphoma group is the significance of mature lymphocytes and clasmatocytes frequently scattered in considerable numbers throughout the

tissue. Three possibilities for their origin must be considered: (1) they are persistent elements of the original nodal tissue; (2) they represent evidences of successful, complete differentiation of tumor cells, and (3) they constitute components of an exudative reaction to the products of disintegrating tumor cells. It is probable that each of these possibilities is, on occasion, an actuality and all efforts at interpretation must be guarded.

A problem offering equal difficulty is the correct interpretation of multinucleated cells in these tumors. On the one hand is the knowledge that multinucleated "tumor giant cells" may occur in almost all types of malignant, and in a few benign, neoplasms. On the other hand is their peculiar frequency in Hodgkin's disease. We have felt that occasional multinucleated cells do not necessarily predicate a classification as Hodgkin's disease.

Cytologically, two broad lines of division in malignant lymphoma have long been recognized: Hodgkin's disease with its complicated structure, described independently by Sternberg [7] and by Reed;[8] and the simpler proliferative conditions of the lymphocytic series of cells variously termed, according to their distribution, lymphatic leukemia, aleukemic leukemia and lymphosarcoma. More recently, Roulet [9,10] has added a third type, named by him "Retothelsarkom" and generally entitled in this country, reticulum cell sarcoma. The justification of such further subdivision was immediately attested by the prompt and wide acceptance of the term throughout the world. Whether or not this last group represents a single entity may, however, legitimately be doubted in view of the wide discrepancies in the descriptions and theories of its origin which various authors have offered. A complete bibliography would be profitless, but let us examine a few examples. Ewing [11] implied that reticulum cell or large round cell sarcoma is a tumor composed of cells less mature than the lymphocyte derived from the germinal centers or pulp cords. Klemperer [12] stated that the reticulum cell is totipotential and similar to cells observed in the embryonic mesenchyme. Medlar [13] used the expressions reticulum cell and stem cell interchangeably. Rhoads [14] derived the tumor from elements of the phagocytic cell series, a view followed by Parker and Jackson.[15] The latter authors clearly distinguished reticulum cell sarcoma from the malignant type of Hodgkin's disease, but Callender [1] stated that reticulum cell sarcoma is Hodgkin's sarcoma. Roulet, himself, included under the heading of Retothelsarkom a group of lesions with a morphologic range which, judging from his descriptions and his illustrations, extends almost from one extreme to the other of the malignant lymphomas.

It is apparent that various authorities regard the type cell of this

tumor (1) as an immature cell of the lymphocyte series, (2) as a pluripotential cell of variously assumed potentialities of development including the formation of lymphocytes, phagocytes and of reticulum and collagen, and (3) as a relatively well differentiated cell of the monocyte or clasmatocyte series. When such variation of opinion exists it seems probable that the individual authors cited cannot be describing the same tumor; that, in fact, reticulum cell sarcoma, as the term is at present used, represents not an entity but a blanket-term covering all primary tumors of lymph nodes not otherwise classifiable. Our own observations lead us to believe that so-called reticulum cell sarcoma must be divided into two types: (1) tumors composed of relatively well differentiated wandering cells with phagocytic propensities resembling monocytes or clasmatocytes, and (2) tumors made up of highly undifferentiated, presumably pluripotential cells which we have chosen to call stem cells. The justification of such a step will, we hope, be made apparent in the descriptive sections to follow. For those who hesitate to accept such identification, this "stem cell" group may simply be regarded as consisting of those tumors of the lymphocytic series too undifferentiated to classify. With this single exception our classification does not deviate from generally accepted cytologic lines.

1. Stem Cell Lymphoma

There is general agreement concerning the existence in lymph nodes of an undifferentiated cell of mesodermal origin which, as a result of unknown stimuli, develops the ability to differentiate into various forms of blood cells.[16-19] A structurally indistinguishable cell is to be found throughout the remainder of the hematopoietic system. The extent of its pluripotentiality in lymph nodes and elsewhere is still unsettled and cannot be discussed in this paper. Such cells have variously been termed lymphoidocyte,[20] primitive blood cell,[21,27] hemohistioblast,[22] hemocytoblast,[23] reticular [24,25] and reticulum cell [12,13,26] and common lymphoid stem cell.[27] The last term seems to us simplest and most appropriate and will be used throughout the article.

The lymphoid stem cell is a large cell, 15 to 35 μ in diameter, with variably abundant, pale-staining, amphophilic cytoplasm possessing a poorly defined, often imperceptible outline. When closely packed, as in the center of a hyperplastic follicle, the cells may appear fused as if constituting a syncytium. Where the cells are more discrete, intercellular bridges are sometimes noted. The nucleus is large, two to four times that of a normal lymphocyte, is usually round and its border is thin but distinct. Chromatin is extremely delicate, irregularly distributed and generally lacks points of condensation. There is, however,

usually a single prominent vesicular nucleolus. We have been able to observe no unequivocal evidence of reticulum formation by these cells under either normal or neoplastic conditions.

Cells of this type, or at any rate cells morphologically indistinguishable from them, are found in small numbers in all tumors of the lymphoma group, just as they are present in normal lymphoid tissue. In the majority of lymphomas, however, they are so rare as to escape notice unless specifically searched for. In a limited group they constitute the predominant element and it is for this group that we have proposed the name "stem cell lymphoma."

Tumors of this type occur in two forms clearly described by Ehrlich and Gerber [24] under the heading of reticular and intermediary types of lymphosarcoma. In one the neoplasm appears to consist of homogeneous syncytial sheets (Fig. 1) and in the other of discrete cells (Fig. 2). In general, however, the fundamental similarity is so great and the two forms of growth appear simultaneously with sufficient frequency to warrant combining them as a single histologic unit. In the discrete cell type (Fig. 4) the individual cells conform closely to the description given for the stem cell of the normal lymph node, tending, however, to be larger and in a given lesion to be very uniform in size. One variation is the usual presence of a single, large, densely basophilic nucleolus. The cytoplasm is homogeneous, poorly outlined and occasionally, but rarely, exhibits particulate phagocytosis. The syncytial type which occurs much less frequently exhibits exactly similar cytologic characteristics but lacks almost entirely the separation of one cell from another (Figs. 1 and 3).

Moderate numbers of lymphocytes and monocytes are noted in some of the tumors, very few in others, particularly those of the syncytial type. Fibrosis is observed in a few of the cases but is an unusual occurrence. Reticulum is comparatively sparse and its distribution is irregular in all the tumors of this group, particularly in those of the syncytial type.

2. *Clasmatocytic Lymphoma*

In contrast to the preceding group, with which, in our estimation, it has been widely confused, the cells in these tumors simulate more or less closely normal clasmatocytes or monocytes. Distinction between the last named types of cells has been somewhat overemphasized.[28-30] We are in agreement with the belief [31,32] that they are closely related and often indistinguishable from each other. They tend to be smaller than the stem cells of the preceding group but are distinctly larger than lymphocytes, varying from 14 to 22 μ in diameter. The cytoplasm is abundant, generally eosinophilic, and its borders, though distinct, tend

to be irregular in outline, suggesting ameboid propensities (Fig. 5). Phagocytic qualities are marked and, though usually limited to particles, engulfment of whole cells occurs. Nuclei are frequently eccentric in position; a few are round, more are oval and still others are reniform or even horseshoe shaped (Fig. 6). Chromatin forms a moderately fine network and nucleoli are rarely evident. The rate of growth and degree of differentiation varies over considerable limits, but in most tumors sufficient numbers of apparently mature monocytic or clasmatocytic elements are present to aid materially in identification.

In the less differentiated examples the distinction from stem cell lymphoma is difficult and in some neoplasms, which by all other criteria appear to belong in this group, the presence of multinucleated cells makes confusion with Hodgkin's sarcoma possible. Small numbers of lymphocytes are occasionally found sparsely and irregularly sprinkled, presumably evidence of exudative reaction. Reconstruction of reticulum is relatively scanty. Scattered fibrils without regular network are observed in most lesions. There is little evidence to support the contention that fibrils arise from the tumor cells.[1,9,33] A few of the specimens exhibit a coarse latticework of collagen but none is actually scirrhous in appearance.

3. *Lymphoblastic Lymphoma*

The predominant cell in these lesions is a lymphoblast. It is a spherical cell which, however, frequently exhibits irregularity of outline with pseudopod-like protuberances. It is larger than a mature lymphocyte, varying ordinarily within the range of 10 to 20 μ in diameter, and possesses a relatively uniform, narrow, basophilic rim of cytoplasm (Figs. 7 and 8). The nucleus is likewise larger than that of the lymphocyte, is centrally placed, round or slightly indented. The nuclear border is sharp, the chromatin rather evenly distributed and much less clumped than in the mature elements, giving the nucleus as a whole a vesicular appearance. Nucleoli are infrequently observed. As would be expected in a relatively undifferentiated tumor, mitotic figures are usually numerous.

Though the lymphoblast is always the predominant cell in specimens included in this group, stem cells are present in moderate numbers and in some cases considerable numbers of lymphocytes can be found. The nodal architecture is characteristically obscured. Many specimens exhibit a homogeneous appearance as the result of the uniformity of component cells and the even distribution of the reticulum framework. The majority, however, are somewhat irregular in appearance because of incomplete stromal revision.

4. Lymphocytic Lymphoma

The predominating cell in this lesion is indistinguishable from a normal lymphocyte (Fig. 10). Stem cells and lymphoblasts are present in small numbers scattered indiscriminately, but usually are too infrequent to cause diagnostic difficulties. Mitotic figures are sparse and no multinucleated cells appear. Nodal architecture, including sinus and follicle structure, is characteristically obscured by the relatively uniform infiltration of small lymphocytes (Fig. 9). As in the preceding group, however, stromal revision is frequently incomplete and some irregularity remains as the result of persisting portions of uninvolved nodal tissue or of small focal collections of less mature cells. The nodal capsule is usually intact but invasion may occur. Extension into perinodal tissues simulates closely the appearance of the original nodal lesion.

Leukemia. Upon completion of both the clinical and histologic studies an attempt was made to predict the presence or absence of clinical leukemia on the basis of the nodal morphology in both this group and the lymphoblastic lymphomas. No criterion for distinction held. Nodes with apparent blood-vessel invasion were obtained from patients without leukemia, and many with pericapsular invasion or large invasive tumors simulating Kundrat's lymphosarcoma [34] were accompanied by leukemia. It was necessarily concluded that it is impossible to distinguish the leukemic from the non-leukemic lesion by means of lymph node morphology.

5. Hodgkin's Lymphoma

Hodgkin's disease (the eponymic terminology is still more widely accepted than any of the suggested synonyms such as malignant lymphadenoma or lymphogranuloma) constitutes the commonest and most readily recognizable lesion among the malignant lymphomas. Its range of clinical and histologic variation is so great that subdivision appears necessary and the two terms, Hodgkin's lymphoma and Hodgkin's sarcoma, have been employed to designate the two divisions which we have recognized. Of the utility of a third subdivision, Hodgkin's granuloma,[35,36] we are still unconvinced. In contrast to the preceding groups, in each of which proliferation of a single type of cell completely dominates the picture, Hodgkin's lymphoma is essentially polycellular (Fig. 11). The majority of the constituents; *i.e.*, granulocytes (usually eosinophilic but frequently neutrophilic), lymphocytes, plasma cells, clasmatocytes and fibroblasts, are the usual components of various inflammatory reactions. Other elements, however, the only elements, moreover, which can be regarded as pathognomonic and whose pres-

ence is universally admitted essential for diagnosis of the lesion, are not found in any inflammatory process of established etiology. They consist of stem cells, frequently indistinguishable from those of "stem cell lymphoma," which tend strongly to develop large multilobed or multinucleated forms (Fig. 12). It is important from the diagnostic point of view, moreover, to recall that both Sternberg [7] and Reed [8] noted mononucleated as well as multinucleated "giant cells" in this disease. Although their names are commonly applied to the multinucleated forms, cells with single or mirror-image double nuclei are equally characteristic of the process.

The specific cells are quite variable in size, ranging from 10 to 40 μ, or more, in diameter. The cytoplasm is abundant and its staining reactions, though usually intense, are variable, ranging from acidophilia to basophilia in different cases, though relatively constant in the same case. Single nuclei are large and round, oval, or slightly indented; they are vesicular in appearance and contain chromatin without characteristic distribution. Within the majority, single nucleoli are found which differ from those noted in the stem cell tumor in that they are not densely stained but are actually vesicular in appearance.

In the multinucleated forms the nuclear masses may show narrow connecting bridges or may be entirely discrete. In either case large nucleoli are usually evident in each nuclear mass and the nuclear masses are characteristically dissimilar in size and shape. Even where each of the many nuclei is discrete they tend to overlap and remain clustered in the center of the cell. Unipolar mitotic figures are easily found and multipolar mitoses are not unusual. Mitotic activity is unusual, however, in any of the other cells composing the lesion.

A great variety of other elements are always to be observed in varying frequency and abundance. Although general trends are apparent, no constant chronologic sequence can be made out for the appearance of each type of cell. It is necessary to remember that the disease does not run a simultaneously parallel course in each node of a given patient. Early lesions may be evident in one region while the process is advanced in another area. The approximate age of an individual lesion may be roughly estimated from the histologic appearance but not the duration of the disease in the patient.

Lymphocytes are always present. They are most abundant in the early stages and their relative numbers diminish later with progression of fibrosis or with dedifferentiation of the lesion. Plasma cells are frequently encountered although they are more evident in advanced stages. In no other lymphoma subgroup do they appear with an equal degree of frequency.

Granulocytes, both eosinophilic and neutrophilic, are usually but not invariably present. They bear no constant relation to the occurrence of necrosis. Eosinophils are more common and in some cases constitute the predominant cell. Monocytes and clasmatocytes are present in variable numbers. Hodgkin's lymphoma exhibits more tendency to focal necrosis than the other types of lymphoma and in lesions showing this phenomenon phagocytes are quite numerous. In a few cases, however, in the absence of evident necrosis, phagocytic cells were so abundant that distinction from clasmatocytic lymphoma was difficult.

Collagen production is roughly proportionate to the duration of the lesion and is comparatively scanty in the early phases. This stage, however, is transient; and wavy, non-argentophilic, interlacing strands soon appear, progressing steadily by fusion of the strands until broad fibrous septa separate the foci of cellularity into islands in the scirrhous tumor (Fig. 13). Still later, if the disease is sufficiently prolonged, the entire node becomes replaced with dense fibrous tissue. Nodes of this type are found as frequently in areas not subjected to roentgen therapy as they are in regions so treated. Such a lesion is not then necessarily significant of the effect of irradiation.

6. Hodgkin's Sarcoma

It has been stated that Hodgkin's lymphoma may, after following a comparatively benign, prolonged course, undergo both clinical and histologic transformation into a rapidly progressive, highly malignant tumor.[35,37,38] Only a few cases in this series have exhibited histologic metamorphosis of this type (Table I), although many clinical histories have suggested that such a change has taken place. Most of the cases of Hodgkin's sarcoma have shown the characteristic morphology of this type at the outset.

Hodgkin's sarcoma retains the fundamental background of Hodgkin's lymphoma, the basic cell being the tumor stem cell or Sternberg-Reed cell. The peculiar difference is the marked preponderance of these cells over all other elements comprising the tumor (Fig. 14). Characteristically, the lesion consists of large numbers of these cells without syncytial relations, exhibiting marked variability in size and nuclear configuration (Fig. 15). Multinucleated cells predominate and mitotic figures are very numerous. Lymphocytes, plasma cells and eosinophils, though present, are minimal in numbers and fibrosis rarely proceeds beyond the early background of strandlike collagen. Densely scirrhous tumors do not occur. Monocytes and clasmatocytes are present, many of the latter attaining features simulating neoplastic change. In certain of the tumors this is so striking that distinction from undifferentiated forms of clasmatocytic lymphoma becomes difficult.

7. *Follicular Lymphoma*

This unusual subgroup was originally segregated as "giant lymph follicle hyperplasia" by Brill, Baehr and Rosenthal [39] but later classified as a manifestation of malignant lymphoma.[40, 41] It has been implied by Ewing [11] and Jackson [36] that it constitutes an inconstant borderline group, evidently a developmental phase of variable duration ultimately becoming one of the other types of lymphoma. Callender [1] attributed a relatively high degree of malignancy to this type while Symmers at first [42] did not believe that the condition was neoplastic at all and later [43] conceded this point with considerable reservation. Although there is evidence that with a sufficiently prolonged course the structural arrangement of the follicular lymphomas may eventually approximate that of one of the other types of lymphoma, there can be little question relative to either the individuality or the ultimate malignancy of the lesion.[44]

Fundamentally, it manifests itself by complete replacement of normal lymph node architecture by multiple follicle-like nodules of varied size and approximation (Fig. 16). These are also present in regions of extranodal invasion. The structure of nodal reticulum is characteristically revised. Trabeculae are obscured. Surrounding each follicular nodule the reticulum meshwork is obviously distorted and condensed by the expanding follicle, and the normally loosely arranged network with broad, polygonal pulp spaces (Fig. 19) becomes compressed and the inter-reticular spaces markedly elongated and narrowed (Fig. 21). Such stromal rearrangement does not occur in ordinary hyperplasia of lymph nodes and may therefore be considered to be of some diagnostic value. Despite this condensation of interfollicular fibrils, actual fusion with the production of collagen does not occur. The sinuses are evidently obscured by displacement of condensed fibrillar material into them. The sparse, stretched attachments of follicles to the surrounding framework allow for separation of the follicles from surrounding tissues in the process of sectioning. This seeming "cracking off" of follicular from nodal substance is due to artefact but is sufficiently constant to serve as a differentiating criterion (Figs. 16 and 18). It is observed relatively uncommonly in other lymph node conditions.

In sections stained in the usual fashion with phloxine and methylene blue several types of follicular nodules are observed. These vary in different cases, apparently with the duration of the ailment, but are type-constant in a given specimen. Detailed description of this phenomenon has been recorded elsewhere [44] and will not be further elaborated here. Although these intrafollicular variations represent features characteristic of this form of lymphoma, the general morphologic

peculiarities deserve greater emphasis in a presentation of this nature.

Significant numbers of multinucleated cells do not appear, nor are there evidences of necrosis or inflammatory exudation. Invasive qualities are noted infrequently. In two cases in which focal skin involvement occurred the cutaneous lesions also manifested follicle formation.

Nodal substance intervening between follicles varies considerably with the degree of follicular contiguity. Fusion of two or more impinging follicles occurs occasionally and when these contain "germinal centers" the latter become confluent and the separating rims of small lymphocytes are lost (Fig. 17). In general, the internodular tissue consists of closely packed normal lymphocytes and the compression and obliteration of sinus spaces simulates the appearance of lymphocytic lymphoma. Such similarity is dispelled, however, by reticulum stains and by lower power examination under which the follicular structure becomes obvious.

Since isolated follicle fusion is observed, it is conceivable that, if the course were sufficiently prolonged, fusion would become quite general and the end result indistinguishable from other types of lymphoma. In eight cases in which histologic studies were possible 1 to 11 years after an initial biopsy, transformation into lymphoblastic lymphoma was observed in one case. In all of the others there was persistence of recognizable follicular structure. In these, although a few regions showed coalescence with loss of nodular structure, reticulum stains exhibited the residuum of the characteristic framework described above.

PERSISTENCE OF HISTOLOGIC TYPE

At this point the question of persistence of histologic form in malignant lymphoma in general naturally arises. In 84 cases in this series biopsies were made on two or more occasions, or specimens from both biopsy and subsequent necropsy were available. In 56 of these cases a period of at least 1 month intervened between the two specimens. The average interval in this group was 2.3 years and the range up to 15 years. As shown in Table I, the original histologic structure was maintained in 43, or 76.8 per cent, of the cases. In the remaining 23.2 per cent (13 cases) the lesion became less differentiated in appearance and would, therefore, have been placed in another lymphoma group had the original biopsy not been available. It is believed reasonable to expect this degree of dedifferentiation in any group of malignant tumors followed over an extended period of time. In this particular instance the occasional transition from one form to another is a feature lending credence to the belief that these tumors are essentially of common origin.

Part II. Clinical Studies

If a cytologic classification of the malignant lymphomas such as has been outlined is to have practical value, it should be possible to demonstrate concomitant clinical variations in the subgroups which have been distinguished. An effort will be made to show that this is, within certain limits, the case. Obviously, in a group of diseases so kindred a considerable degree of similarity and of overlapping must

Table I

Retention of Histologic Structure

Type	Average interval between specimens	Number of cases	Unchanged	Dedifferentiation	Differentiation
Stem cell lymphoma	0.25 yrs.	2	2	..	..
Clasmatocytic lymphoma	2.3 yrs.	12	6	6	..
Lymphoblastic lymphoma	0.75 yrs.	4	2	2	..
Lymphocytic lymphoma	1.9 yrs.	9	9	..	..
Hodgkin's lymphoma	1.9 yrs.	20	16	4	..
Hodgkin's sarcoma	0.5 yrs.	1	1	..	..
Follicular lymphoma	5.0 yrs.	8	7	1	..
Total		56	43	13	0

be expected. Certain differential features, nevertheless, became apparent as the material was surveyed which, though of slight importance individually, seemed collectively to delineate a series of fairly distinct clinical pictures.

In 545 cases the clinical records were found to be sufficiently complete to be of value. These were abstracted in detail without reference to histologic classification, then arranged according to histologic group and the clinical data for each subdivision analyzed. Records were complete to the day of death for 413 patients. Of the remaining 132, 21 were in the terminal stage when last seen and were presumed to have died shortly after the last observation. Fifty patients were lost and 61 are still alive, the majority of them under observation in the tumor clinic of this hospital.

Age at Onset

The age at which the first presumably relevant symptom was noted has been recorded as the age at onset. Undoubtedly inaccurate as such a method is, the errors should approximately balance and, consequently, comparisons between the various subgroups should be valid. Figures for the average and median ages of onset of each of the subgroups appear in Table II. For the entire group the average was 42.5 years and the median 44. The most notable deviation from these

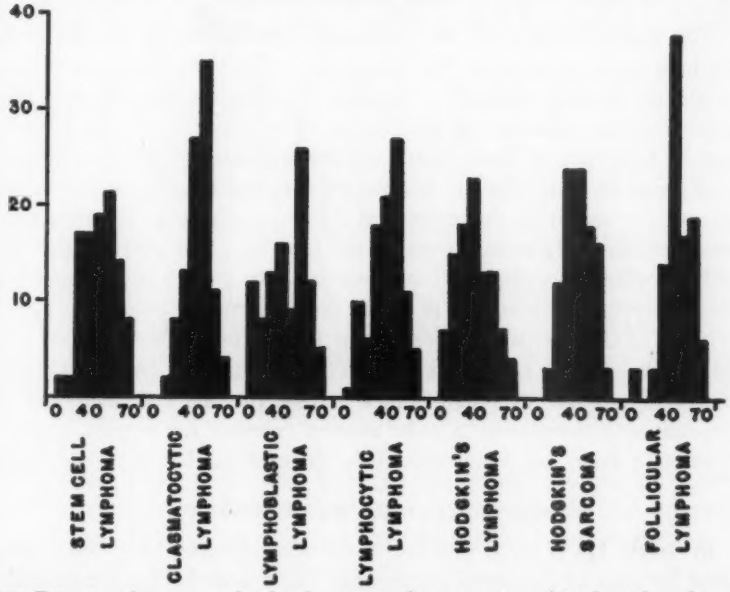
levels occurred in Hodgkin's lymphoma where both figures indicate a tendency for the disease to appear nearly a decade earlier than with any of the other subtypes. Slight deviations in the other direction above the mean level are apparent in the clasmatocytic and follicular subgroups.

TABLE II

Age at Onset (Years)

Type	Males		Females		Total	
	Average	Median	Average	Median	Average	Median
Stem cell lymphoma	47	50	43	41	46	48
Clasmatocytic lymphoma	45	43	52	55	49	49
Lymphoblastic lymphoma	44	43	37	38	41	45
Lymphocytic lymphoma	45	49	44	44	44	47
Hodgkin's lymphoma	37	30	34	34	36	34
Hodgkin's sarcoma	44	44	46	43	45	43
Follicular lymphoma	51	49	49	42	50	46

More convincing evidence of characteristic group variation is provided in Text-Figure 2 in which age distribution is charted. Although examples of each of the specific types were met in every age group, it is apparent that age distribution is more or less characteristically conditioned by histologic type. For all types except Hodgkin's lymphoma the disease most frequently became evident in the fifth and sixth



TEXT-FIGURE 2. Age at onset by decades, expressed as percentage of total number of cases in each subgroup.

decades, the latter usually leading by a small margin. Ordinary Hodgkin's, in contrast, showed maximal incidence in the third and fourth decades.

Only three types of lymphoma occurred with significant frequency in the first two decades. Well in the lead in development in youth was Hodgkin's lymphoma in which 24 per cent of the cases appeared before the age of 20. Most nearly comparable was the lymphoblastic type with 18 per cent of cases in this age period. In third place with 11 per cent below 20 years of age was lymphocytic lymphoma which proved unique in showing a higher percentage of occurrence in the second decade than in either the first or third, a finding reinforced by the similar observation of Jackson.[35]

At the other end of the scale come the clasmatocytic and follicular types. Both of these were extremely infrequent in youth and relatively uncommon in the twenties and thirties as shown by the fact that only 22 and 21 per cent respectively occurred below 40 years of age. Somewhat intermediate distribution was recorded for the stem cell type and Hodgkin's sarcoma. Here, too, cases were rare before 20 years of age but enough appeared in the third and fourth decades to give incidences of 38 and 42 per cent respectively below the age of 40.

Sex Incidence

The greater frequency of malignant lymphoma in the male sex has long been recognized. For the entire series the proportion of men to women affected was 2.2:1. Hodgkin's lymphoma was the only subgroup strictly adhering to this mean. The other types, as may be seen by reference to Text-Figure 3, showed greater male predominance ranging up to 3 to 1 in the lymphocytic, lymphoblastic and stem cell groups whereas in the follicular and clasmatocytic types the frequency was approximately equal in males and females. It is possible that the lack of evident sex difference in these last two groups is dependent on the relatively advanced age at which they commonly occur.

None of the comparative variations in age incidence or duration of the disease in the two sexes recorded in Tables II and IV appears great enough to have significance unless the marked incidence of clasmatocytic lymphoma in older women (median for women 55 years against 43 years for men) should be substantiated in a larger series.

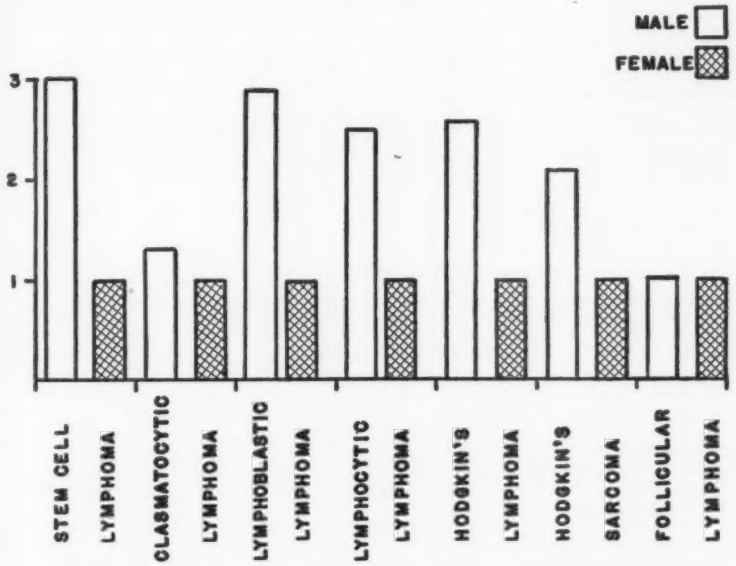
GENERAL CLINICAL MANIFESTATIONS

In Table III a large number of clinical features have been tabulated for each of the seven subgroups. They have been selected either because they appeared to offer opportunities for noteworthy compari-

sons or contrasts, or because they have received widespread comment in the literature. In the descriptive section to follow, emphasis has been placed upon manifestations which reflect differences between the various subgroups.

Enlargement of Lymph Nodes and Spleen

Palpable, visible, or presumptive lymph node enlargement was present in well over 90 per cent of the subgroups. Among only the patients with clasmatocytic and stem cell lymphoma were there 20 per cent



TEXT-FIGURE 3. Sex incidence expressed in terms of relative proportion.

without evidence of disease of lymph nodes. Peripheral lymph nodes were enlarged most frequently in Hodgkin's, lymphocytic and follicular lymphoma and retroperitoneal nodes were outstandingly prominent in the follicular type. Mediastinal nodes were enlarged in roughly the same proportions in each group although they were noted less frequently in the clasmatocytic and stem cell lymphomas. Splenomegaly was present most frequently with lymphocytoma (56 per cent) and slightly less frequently with Hodgkin's, lymphoblastic and follicular lymphoma (34 to 46 per cent). Splenic enlargement was infrequent in Hodgkin's sarcoma and in clasmatocytic and stem cell lymphoma (14 to 23 per cent).

TABLE III

Distribution of Clinical Observations According to Type of Lymphoma

	All types	Stem cell	Clasmatocytic	Lymphoblastic	Lymphocytic	Hodgkin's	Hodgkin's sarcoma	Follicular
Miscellaneous data								
Number of cases	545	42	64	76	118	174	33	38
Age of onset (average)	42.5	46	49	40	44	36	45	50
Onset under 20 years	13%	4%	2%	20%	11%	22%	3%	3%
Onset under 40 years	44%	38%	23%	49%	35%	63%	39%	20%
Onset over 40 years	56%	62%	77%	51%	65%	37%	61%	80%
Average total duration (yrs.)	2.9	1.7	2.1	1.4	3.3	4.2	1.8	5.6
Mortality in initial 2 years	53%	80%	76%	80%	45%	34%	89%	13%
Five-year survivals	22%	14%	11%	3%	25%	29%	7%	53%
Ten-year survivals	4%	3%	2%	0	2%	8%	3%	16%
Radioresistant cases	9%	12%	8%	21%	3%	8%	20%	5%
Proportion, male: female	2.2	3.0	1.3	2.9	2.5	2.6	2.1	1.0
Physical examination								
Pruritus	14%	7%	11%	12%	23%	21%	18%	0
Purpura	8%	5%	1%	13%	20%	4%	3%	0
Ulcerative phenomena	24%	42%	31%	29%	23%	17%	39%	4%
Obstructive phenomena	35%	49%	49%	39%	28%	26%	33%	58%
Hydrothorax	21%	14%	17%	26%	13%	25%	30%	31%
Ascites	18%	14%	15%	15%	13%	20%	21%	37%
Fever	40%	29%	25%	42%	43%	49%	59%	12%
Intermittent fever	7%	0	3%	3%	4%	16%	15%	0
Stigmata of tuberculosis	15%	2%	17%	10%	15%	19%	15%	20%
Lymph node involvement	91%	79%	80%	93%	91%	95%	94%	100%
Retroperitoneal lymph nodes	49%	30%	40%	50%	46%	50%	48%	79%
Mediastinal lymph nodes	45%	30%	23%	45%	40%	61%	54%	37%
Splenomegaly	40%	14%	23%	46%	56%	45%	18%	34%
Gastro-intestinal involvement	13%	27%	20%	14%	11%	9%	24%	6%
Genito-urinary involvement	10%	0	8%	15%	19%	5%	15%	4%
Pulmonary involvement	9%	9%	3%	7%	8%	12%	15%	4%
Cutaneous involvement	20%	17%	16%	21%	26%	20%	24%	4%
Discrete bone involvement	13%	7%	23%	15%	7%	16%	15%	4%
Diffuse bone marrow involvement	10%	5%	5%	20%	21%	5%	3%	2%
Hematologic data								
Anemia	40%	29%	17%	54%	41%	44%	33%	29%
Leukocytosis	44%	25%	31%	52%	59%	45%	50%	15%
Thrombocytosis	18%	15%	33%	30%	2%	18%	47%	0
Thrombocytopenia	31%	14%	10%	40%	58%	27%	3%	25%
Monocytosis	21%	15%	22%	12%	9%	35%	33%	6%
Eosinophilia	8%	10%	3%	2%	5%	15%	3%	3%
Lymphocytosis	31%	13%	16%	62%	70%	14%	7%	18%
Lymphocytopenia	8%	0	2%	2%	4%	19%	13%	0
Atypical cells	27%	7%	14%	60%	33%	25%	20%	6%
Leukemia	9%	2%	5%	15%	23%	2%	3%	3%
Subleukemia	8%	0	0	23%	25%	0	0	0

Fever

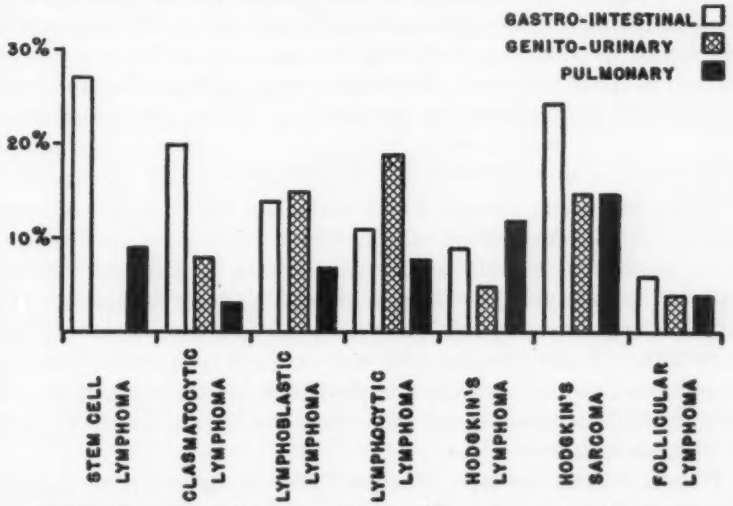
Febrile manifestations (fever of 101° F. or higher) occurred at some time during the course of the disease in cases from all the subgroups (Table III). It was rare in the follicular type (only 12 per cent), relatively uncommon (25 and 29 per cent) in the clasmatocytic and stem cell groups, appeared in 42 and 43 per cent of the lymphocytic and lymphoblastic cases, in half the Hodgkin's lymphomas and was most frequent in Hodgkin's sarcoma (59 per cent). Intermittent fever of the Pel-Ebstein type occurred with significant frequency (16 per cent) only in the two Hodgkin's types and was noted very infrequently among the others.

Cutaneous Involvement

Generalized cutaneous infiltration, scattered nodular lesions of the skin, or a combination of both of these appeared in 16 to 26 per cent of the cases in all groups except the follicular lymphomas, among which cutaneous lesions were unusual. Pruritus was recorded in cases in each group except that of follicular lymphoma. Although individuals with skin lesions were prone to suffer from this symptom, it bore no constant relationship to the presence of evident cutaneous disease and was not believed to be of diagnostic value.

Visceral Involvement

There appeared to be a significant group variation with regard to visceral predilection (Text-Figure 4). The gastro-intestinal tract was



TEXT-FIGURE 4. Visceral involvement by percentage of cases in each subgroup.

more often involved in patients with Hodgkin's sarcoma, stem cell and clasmatocytic lymphoma. These lesions were very unusual in follicular lymphoma. Tumor infiltration of the genito-urinary apparatus was most common among the lymphocytomas, less common in lymphoblastic lymphoma and Hodgkin's sarcoma and rare in the other types. Lymphomatous lesions of the lungs generally exhibited the histologic features of one or the other forms of Hodgkin's disease. Pulmonary foci were comparatively rare among the other subgroups.

Obstructive phenomena as the result of compression of vascular or visceral channels were particularly striking in follicular lymphoma. There was, in general, less evidence of obstruction in the clasmatocytic and stem cell lymphomas and considerably less among the remaining subgroups. Ulceration secondary to neoplastic infiltration of skin or mucous membranes occurred most frequently with stem cell lymphoma and Hodgkin's sarcoma.

Peripheral edema and ascites appeared in over one-third of the cases of follicular lymphoma and were much less common in the remaining groups. There was no differential significance in the frequency of occurrence of hydrothorax. Chylous effusion was observed in 11 per cent of the follicular lymphomas and was rarely encountered in any other type of the disease.

Bone Lesions

Isolated skeletal lesions, in many instances solitary manifestations of the disease, were recorded in one quarter of the patients with clasmatocytoma. They appeared less frequently in Hodgkin's and lymphoblastic lymphoma and Hodgkin's sarcoma and were distinctly unusual in the other types. The presence of pathologic fracture varied directly with the frequency of discrete bone lesions (Text-Figure 5).

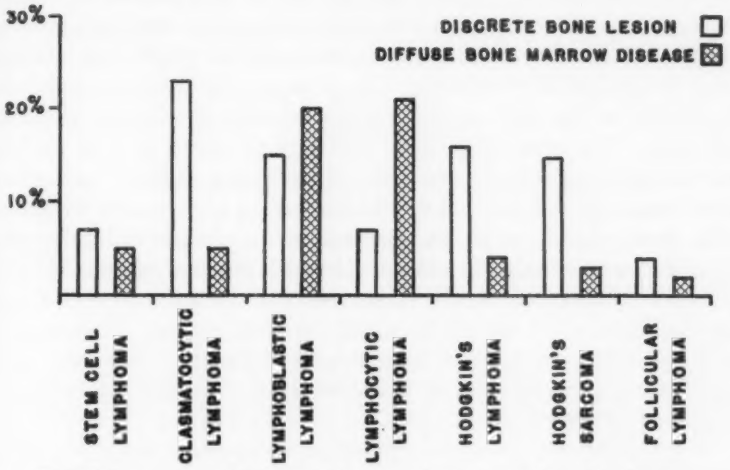
HEMATOLOGIC OBSERVATIONS

Of the nonspecific changes in the peripheral blood, anemia was the most important. A red blood cell count of 3.5 million or less was present in one-half to one-third of the patients with lymphoblastic, Hodgkin's and lymphocytic lymphoma and Hodgkin's sarcoma in descending order of frequency. Anemia was very much less common among the cases with follicular, clasmatocytic and stem cell lymphoma. Profound anemia was recorded most often in the lymphoblastic and lymphocytic lymphomas with leukemic manifestations and in the terminal stages of Hodgkin's disease.

Platelet estimations were infrequently made and were too inaccurate for critical analysis. Thrombocytosis occurred with significant

frequency only among the patients with Hodgkin's sarcoma and thrombocytopenia among those with lymphocytic and lymphoblastic lymphoma. Purpura was noted more commonly in the two last-named groups.

A leukocytosis exceeding 12,000 per cmm. was observed in 50 to 60 per cent of the patients with lymphocytic and lymphoblastic lymphoma and Hodgkin's sarcoma. It was less common with Hodgkin's,



TEXT-FIGURE 5. Bone involvement by percentage of cases in each subgroup.

clasmatocytic and stem cell lymphoma, and was very unusual among the cases of follicular lymphoma. Leukopenia with white cells numbering less than 5,000 per cmm. was noted in 11 to 16 per cent of the patients suffering from lymphocytic, lymphoblastic and Hodgkin's lymphoma. It was rare in other types of the disease.

Monocytes in excess of 10 per cent were recorded in one-third of the cases of Hodgkin's lymphoma and sarcoma and one-quarter of those with clasmatocytomas, but rarely in the remainder. Markedly diminished monocyte values occurred with the lymphocytic and lymphoblastic lymphomas.

Lymphocytosis was observed in 62 to 70 per cent of the cases with lymphocytic and lymphoblastic lymphoma. Lymphocytopenia appeared in only 19 per cent of the Hodgkin's lymphomas and 13 per cent of the Hodgkin's sarcomas. The remaining groups generally exhibited normal values. Variations in the number of eosinophils were considered to be lacking in pertinent diagnostic value. Eosinophilia was not a prominent feature in Hodgkin's disease.

Atypical cells, "tumor cells," unidentified cells and "blast" forms appeared in the peripheral blood of almost two-thirds of the cases of lymphoblastic lymphoma, one-third of those with lymphocytoma and one-quarter of those with Hodgkin's lymphoma and sarcoma. In only 6 to 14 per cent of the remainder were such phenomena apparent.

Leukemia

Peripheral blood pictures characteristic of leukemia as commonly defined occurred at some time during the course of the disease in 48 per cent of the cases with lymphocytoma and in 38 per cent of those with lymphoblastic lymphoma. They were infrequent but were encountered among each of the remaining subgroups except the stem cell type. The leukemic pictures were susceptible to division into the various phases commonly ascribed to this disease. These consisted of: true leukemia with the white cells numbering over 30,000 associated with an absolute increase and preponderance of cells of the lymphocytic series; sub-leukemic leukemia in which the total number of white cells fell between the normal range and minimal leukemic levels; and finally, aleukemic leukemia in which the total number of white cells was well below the minimal normal level of 5,000 but in which again there was a predominance of lymphocytes or "atypical" or immature lymphoid forms.

The significance of such a classification appears dubious when a series of cases is followed over a considerable period of time. Fifty patients who exhibited some phase of leukemia during their course were selected for analysis on the basis that two or more blood studies were recorded at extended intervals during their disease. Of these, 13 patients were non-leukemic when first observed but developed leukemia later in the course, six were leukemic at the initial observation but showed normal blood pictures prior to death and two other patients were leukemic at the beginning and at the end of the disease but were non-leukemic in the interval. Forty-two per cent of this group, then, exhibited marked relapse into, or remission from, leukemia. An additional 38 per cent showed a variety of shifts among the aleukemic, subleukemic and leukemic phases without reverting to normal. In only 20 per cent of the 50 patients was the blood picture entirely constant throughout the course of the disease.

Since, as has been shown in the first portion of this study, there is no difference in the histologic background of the leukemic and the non-leukemic cases, it appears evident that the development of leukemia must be regarded as an incidental occurrence in the disease—simply an overt manifestation of the underlying process. Actually nothing

is known relative to the mechanism of the delivery of the abnormal cells into the blood stream or concerning the abrupt changes in the blood picture so frequently observed.

The development of leukemia was of some prognostic import. Seventy-seven patients with lymphoblastic or lymphocytic lymphoma, with leukemia recorded at least once, showed an average life expectancy of about 1 year less than those without leukemic manifestations. Its presence at the onset or its development during the course generally implied a poorer prognosis, particularly if the leukemic cells were immature or bizarre in appearance and the causative lesion was of the lymphoblastic type.

"Lymphosarcoma"

Malignant lymphoma in all its varieties tends to be a generalized disease, or to become such so rapidly that it is the exceptional case which is observed in the localized stage. From clinical study it is scarcely possible to be certain that the disease is indeed localized, but at the autopsy table isolated lesions are not infrequently met where the most meticulous examination fails to reveal any evidence of generalization. Still more convincing is the experience with surgical resection of lymphomatous tumors which may be followed by survival for many years even without postoperative irradiation; results scarcely interpretable in any other light than as the extirpation of a localized neoplasm.

Localization is not a peculiarity of any cytologic type of lymphoma but is met in all. The frequency, however, with which localized tumors are encountered varies considerably with the histologic type. In 70 of the cases in our series the lesion was limited to a single area at the time of initial examination. Their distribution is recorded in Table IV. Classified according to cytologic type, localization was observed in only 3 per cent of cases of Hodgkin's sarcoma, 6 per cent of Hodgkin's lymphoma, 10 per cent of follicular lymphoma, 11 per cent of lymphocytoma and 13 per cent of the lymphoblastic form. The frequency rose sharply to 26 per cent of stem cell and 33 per cent of clasmatocytic lymphoma.

It is of interest that over half of these lesions appeared in non-lymphoid tissues, most frequently in bones and in the gastro-intestinal tract, but occasionally in areas in which even isolated lymph follicles are not found under normal circumstances (*i.e.*, the subcutaneous fat, the bladder and the cervix of the uterus). Many of these tumors grow in a frankly invasive and destructive manner and metastases may occur in many organs without diffuse involvement of the lymphoid structures or the bone marrow.

TABLE IV

Initially Localized Malignant Lymphoma (Lymphosarcoma; Kundrat)

No.	Type	Location	Treatment	Duration	Ultimate distribution	Result	Autopsy
1	Stem cell lymphoma	Mediastinum	X-ray	1.6 years	Local	Dead	
2	Stem cell lymphoma	Cerv. l. n.	Surg., x-ray	10.2 years	Generalized	Dead	
3	Stem cell lymphoma	Stomach	Surg.	0.3 years	None	Dead	
4	Stem cell lymphoma	Stomach	Surg.	2.0 years	None	Dead	
5	Stem cell lymphoma	Thigh	Surg., x-ray	15.7 years	Generalized	Dead	
6	Stem cell lymphoma	Abdom. l. n.	X-ray	1.2 years	Generalized	Dead	
7	Stem cell lymphoma	Dura (cord)	Surg., x-ray	2.2 years	Generalized	Lost	
8	Stem cell lymphoma	Cecum	None	0.4 years	Local	Dead	Yes
9	Stem cell lymphoma	Eyelid	Surg., x-ray	1.9 years	Generalized	Dead	
10	Stem cell lymphoma	Stomach	Surg.	3.0 years	Local	Dead	Yes
11	Stem cell lymphoma	Stomach	Surg., x-ray	0.8 years	Local	Dead	
12	Clasmatocytic lymphoma	Abdom. l. n.	Surg., x-ray	0.5 years	Generalized	Dead	Yes
13	Clasmatocytic lymphoma	Stomach	Surg.	0.3 years	None	Dead	
14	Clasmatocytic lymphoma	Tonsil	X-ray	1.1 years	Generalized	Dead	
15	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	1.3 years	Generalized	Lost	
16	Clasmatocytic lymphoma	Abdominal l. n.	X-ray	1.1 years	Local	Dead	
17	Clasmatocytic lymphoma	Humerus	Surg.	15.9 years	Local	Alive	
18	Clasmatocytic lymphoma	Stomach	Surg., x-ray	7.2 years	None	Alive	
19	Clasmatocytic lymphoma	Dura (cord)	X-ray	0.4 years	Local	Dead	
20	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	3.3 years	Generalized	Dead	Yes
21	Clasmatocytic lymphoma	Stomach	Surg., x-ray	4.9 years	Generalized	Dead	
22	Clasmatocytic lymphoma	Stomach	None	1.0 years	Local	Dead	Yes
23	Clasmatocytic lymphoma	Tibia	Surg.	7.5 years	None	Alive	
24	Clasmatocytic lymphoma	Clavicle	X-ray	4.8 years	Generalized	Dead	
25	Clasmatocytic lymphoma	Femur	Surg., x-ray	4.7 years	None	Alive	
26	Clasmatocytic lymphoma	Stomach	Surg.	5.5 years	None	Alive	
27	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	1.6 years	Local	Dead	
28	Clasmatocytic lymphoma	Femur	X-ray	3.7 years	None	Alive	
29	Clasmatocytic lymphoma	Nasal sinus	X-ray	2.6 years	None	Alive	
30	Clasmatocytic lymphoma	Sacrum	X-ray	1.4 years	Generalized	Dead	Yes
31	Clasmatocytic lymphoma	Ing. l. n.	X-ray	4.1 years	None	Alive	
32	Clasmatocytic lymphoma	Ileum	None	0.3 years	Local	Dead	Yes
33	Lymphoblastic lymphoma	Abdominal l. n.	Surg., x-ray	1.1 years	Local	Dead	
34	Lymphoblastic lymphoma	Jejunum	X-ray	2.3 years	Generalized	Dead	
35	Lymphoblastic lymphoma	Rectum	Surg.	0.3 years	None	Dead	
36	Lymphoblastic lymphoma	Frontal bone	X-ray	2.5 years	Local	Dead	

33	Lymphoblastic lymphoma	Abdominal l. n.	Surg., x-ray	1.1 years	Local	Dead	
34	Lymphoblastic lymphoma	Jejunum	X-ray	2.3 years	Generalized	Dead	
35	Lymphoblastic lymphoma	Rectum	Surg.	0.3 years	None	Dead	
36	Lymphoblastic lymphoma	Frontal bone	X-ray	2.5 years	Local	Dead	
37	Lymphoblastic lymphoma	Tonsil	X-ray	1.7 years	Local	Dead	
38	Lymphoblastic lymphoma	Cerv. l. n.	X-ray	0.4 years	Local	Dead	
39	Lymphoblastic lymphoma	Stomach	Surg., x-ray	3.2 years	Generalized	Dead	
40	Lymphoblastic lymphoma	Parotid	X-ray	1.2 years	Generalized	Alive	
41	Lymphoblastic lymphoma	Mediastinum	None	0.1 years	Local	Dead	Yes
42	Lymphoblastic lymphoma	Mediastinum	None	0.1 years	Local	Dead	Yes
43	Lymphocytic lymphoma	Nasal sinus	Surg., x-ray	7.5 years	Generalized	Dead	
44	Lymphocytic lymphoma	Testis	Surg., x-ray	0.5 years	Generalized	Dead	
45	Lymphocytic lymphoma	Nose	X-ray	2.5 years	Generalized	Dead	Yes
46	Lymphocytic lymphoma	Parotid	None	1.0 years	Local	Dead	Yes
47	Lymphocytic lymphoma	Tonsil	Surg., x-ray	9.0 years	Generalized	Dead	Yes
48	Lymphocytic lymphoma	Cerv. l. n.	Surg., x-ray	3.8 years	Local	Lost	
49	Lymphocytic lymphoma	Prostate	X-ray	4.0 years	Local	Lost	
50	Lymphocytic lymphoma	Stomach	Surg., x-ray	7.0 years	None	Alive	
51	Lymphocytic lymphoma	Femur	Surg., x-ray	8.0 years	Local	Alive	
52	Lymphocytic lymphoma	Dura (cord)	X-ray	0.7 years	Local	Dead	
53	Lymphocytic lymphoma	Intestine	Surg., x-ray	2.4 years	Generalized	Dead	
54	Lymphocytic lymphoma	Mediastinum	None	?	Local	Dead	Yes
55	Lymphocytic lymphoma	Bladder	None	0.3 years	Local	Dead	Yes
56	Hodgkin's lymphoma	Abdominal l. n.	None	1.5 years	Local	Dead	
57	Hodgkin's lymphoma	Back	X-ray	5.5 years	Generalized	Dead	
58	Hodgkin's lymphoma	Cerv. l. n.	Surg., x-ray	8.6 years	Generalized	Dead	
59	Hodgkin's lymphoma	Abdominal l. n.	Surg.	0.6 years	None	Dead	
60	Hodgkin's lymphoma	Parotid	X-ray	9.0 years	Generalized	Dead	Yes
61	Hodgkin's lymphoma	Cerv. l. n.	X-ray	5.9 years	Generalized	Dead	
62	Hodgkin's lymphoma	Abdominal l. n.	X-ray	3.3 years	Generalized	Dead	Yes
63	Hodgkin's lymphoma	Cerv. l. n.	X-ray	8.9 years	None	Alive	
64	Hodgkin's lymphoma	Mediastinum	None	0.2 years	Local	Dead	Yes
65	Hodgkin's lymphoma	Ing. l. n.	None	0.7 years	Local	Dead	Yes
66	Hodgkin's sarcoma	Axillary l. n.	X-ray	1.5 years	Generalized	Lost	
67	Follicular lymphoma	Abdominal l. n.	X-ray	5.5 years	Local	Lost	
68	Follicular lymphoma	Ing. l. n.	Surg.	6.5 years	None	Dead	Yes
69	Follicular lymphoma	Abdom. l. n.	X-ray	2.2 years	Generalized	Dead	
70	Follicular lymphoma	Ing. l. n.	Surg., x-ray	5.5 years	None	Alive	

Observations of this sort have led to the concept of a special form of malignant lymphoma termed by Kundrat [34] "lymphosarcoma" and currently defined [45] as an initially circumscribed lymphoid neoplasm which breaks through its confines and invades neighboring structures by way of lymphatics. Many authors [46-49] believe such a segregation is artificial and merely represents a phase in the course of the disease. The difficulty of maintaining the concept becomes obvious in surveying a large group of cases since "sarcomatous" tumors may even appear in cases of otherwise typical leukemia. Indeed, leukemic blood pictures may develop in cases starting as apparently typical lymphosarcoma, and the confusion is hardly alleviated by the use of terms such as "leukosarcoma" [50] to describe these transitional forms. The absence of detectable bone-marrow involvement cannot be used to rule out leukemia since cases of both lymphatic and myelogenous leukemia have been observed in which marrow replacement by leukemic cells has not occurred.[4, 31, 51] It has been claimed that the type of cell appearing in the blood in leukosarcoma is distinctive and does not resemble that noted in other forms of lymphatic leukemia. This has not been our experience. We have found the character of the circulating cell to be identical with that composing the organic process from which it has arisen.

Of the 70 cases referred to above in which a single localized lesion was present at the outset, 27 ultimately became generalized in character and indistinguishable from other forms of malignant lymphoma. Among the remaining 43 cases, all of which were followed to the time of death or for a period of at least 1 year, 27 showed persistent localization of the process. Twenty-two of these died and only 11 were autopsied so that the evidence of failure of dissemination was purely clinical in 60 per cent. Furthermore, the duration of the disease had been only one-half as long as that in the 27 who developed the generalized condition (2.2 and 4.1 years).

In addition to these 54 cases, 16 showed no evidence of persistent tumor at all. Twelve of these had been subjected to radical surgical excision and 5 died immediately postoperatively. Ten of the 16 were still alive and an autopsy had been made on only 1 of the others. The evidence here also was therefore overwhelmingly clinical and unconfirmed by postmortem investigation.

It seems reasonable to conclude that the condition termed "lymphosarcoma" represents a transient clinical phase and in most instances might be expected to progress into a generalized process if the patient did not succumb at too early a period. Whether or not the dissemination when it does occur is the result of direct extension, metastasis or

independent foci of multicentric origin cannot be stated with any degree of certainty. It is possible that any one or all of these means may be operative.

Duration of the Disease

In a group of diseases which are generally regarded as inevitably fatal, the most practical function of a classification is to aid prognosis. Review of the literature reveals that authors have accepted an extremely discouraging viewpoint regarding sufferers from malignant lymphoma, which is, to a considerable extent, borne out by the median figure of 2.0 years for our entire series. On the other hand, 116 patients, or about 20 per cent, have lived beyond a 5-year period and nearly 10 per cent have survived 8 or more years. It is therefore of importance to determine if consistent variations in survival periods can be correlated with histologic structure.

In surveying our figures it was at once apparent that since many of our patients were still alive, including many of the cases of notably long survival, a false impression would be created if these were excluded. It was therefore decided, somewhat arbitrarily, to include all living cases of 3 or more years' duration. Though such a procedure robs the figures of absolute value—which could only be obtained by the impractical procedure of retiring to a perspective of at least 10 years—it has been uniformly applied and therefore does not affect the validity of the comparative results. Average and median figures for the various subgroups appear in Table V. Inspection at once reveals that four types (lymphoblastic, stem cell and clasmatocytic lymphomas and

TABLE V

Total Duration (Years)

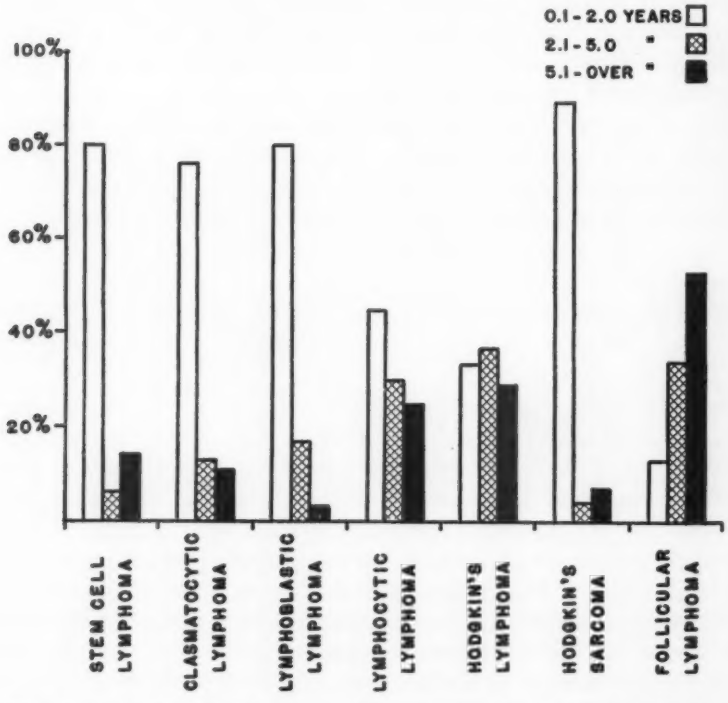
Type	Males		Females		Total	
	Average	Median	Average	Median	Average	Median
Stem cell lymphoma	1.4	1.1	2.5	1.3	1.7	1.1
Clasmatocytic lymphoma	2.3	1.0	1.9	1.2	2.1	1.1
Lymphoblastic lymphoma	1.4	0.5	1.4	0.6	1.4	0.6
Lymphocytic lymphoma	3.4	3.0	3.0	1.9	3.3	2.4
Hodgkin's lymphoma	4.3	3.1	3.7	3.8	4.2	3.2
Hodgkin's sarcoma	1.9	0.9	1.7	0.9	1.8	0.9
Follicular lymphoma	4.6	4.0	6.8	5.6	5.6	5.0

Hodgkin's sarcoma) offer relatively poor prognoses with medians from 0.6 to 1.1 years. The remaining three (lymphocytic, Hodgkin's and follicular lymphomas) tend to run comparatively slower courses with medians of 2.4, 3.2 and 5.0 years respectively.

Early involvement of a vital organ may lead to rapid death even with the relatively benign varieties. Conversely, in even the most

malignant types, occasional instances of notably long survival (8 or more years) are met. These are least frequent in lymphoblastic lymphoma and Hodgkin's sarcoma; they occur with enough frequency in the stem cell and clasmatocytic types to raise the average duration above the median and they are most frequent in the three relatively benign varieties. It is noteworthy that 25 per cent of the cases of lymphocytic, 29 per cent of those with Hodgkin's and 53 per cent of those with follicular lymphoma lived 5 or more years after the clinical onset. On the other hand, only 3 per cent of the lymphoblastomas, 7 per cent of the Hodgkin's sarcomas, 11 per cent of the clasmatocytomas and 14 per cent of the stem cell lymphomas have survived this period (Text-Fig. 6).

Analyses of the cases with the more malignant forms of the disease which had unexpectedly prolonged courses demonstrated that group prognosis apparently was altered by several factors. Patients with primary diffuse skin disease (mycosis fungoides) or single primary lesions of a bone or a viscus (intestine, stomach, thyroid) frequently



TEXT-FIGURE 6. Total duration expressed as percentage of cases in each subgroup.

belied the gloomy outlook predicated by the histologic appearance of the underlying lesion. Cases in which cutaneous, osseous or visceral lesions were secondary phenomena in the course of a generalized process obviously do not belong in this category. Among the outstanding survivals referred to, of which there were 21, 17 showed lesions primary in the regions noted above and the remaining 4 were indistinguishable from the ordinary type of ultimately generalized disease. The longest duration recorded in this series was 23 years, the patient being still alive 21 years since an initial biopsy and 5 years after another, both of which showed classical Hodgkin's lymphoma.

In several of the subgroups the duration was apparently influenced by the age at the onset of the disease. With Hodgkin's lymphoma the survival period was much shorter in those patients in whom the disease was initiated after the age of 50 years. Among the lymphocytomas the life expectancy was better during the middle decades than it was at either extreme of life and it was particularly poor in those few cases occurring in youth. Follicular lymphoma rarely occurred in the young and uniformly offered a relatively favorable prognosis as to period of survival. The four malignant types were relatively uninfluenced by age.

THERAPY

Roentgen therapy has been the only universally accepted means of combating the malignant lymphomas.[45, 52] Indeed, lymphomatous lesions are, for the most part, so strikingly susceptible to this type of irradiation that radiosensitivity has been utilized as a diagnostic criterion for tumors not accessible for histologic study.[53] Despite this common characteristic, individual cases may fail to exhibit any favorable response to such treatment. Such failure has appeared in our experience very rarely among the Hodgkin's, follicular, lymphocytic and clasmatocytic lymphomas, although the last type occasionally required something more than the customary "lymphoma" dosage (600 r.) of deep x-ray therapy to produce subsidence of a lesion. Among the Hodgkin's sarcomas and the lymphoblastic and stem cell lymphomas, however, 20, 21 and 12 per cent, respectively, failed to show any evidence of improvement following irradiation.

It is generally agreed that the benefits of radiotherapy in the malignant lymphomas are but transitory. Moreover, it has even been claimed that although symptomatic relief might be expected, actual prolongation of the course of the disease was unusual.[54, 55] Experience in the clinic leaves the observer with the impression that in many instances roentgen therapy has snatched a patient from immediately impending

death and prolonged his life by months and even years. Acceptable statistical evidence, however, that the average duration of life is prolonged is extremely difficult to accumulate. In a group of diseases where x-ray treatment has become almost automatic once the diagnosis has been established, the collection of an adequate control series is almost impossible. Out of our entire series only 76 patients were found to whom no x-ray treatment had been administered. Most of these were not seen until they were in the terminal stages of their disease, at which time therapy of this type was deemed inadvisable. A few died as the result of surgical intervention, some refused treatment and others were not treated because of failure to recognize the nature of the disease. It is interesting to note that of these 76 patients only 4 survived beyond the average duration established for their respective subgroups. The average total duration of their various illnesses was 0.8 years in contradistinction to the average duration for the entire series of 2.9 years. The two series are by no means strictly comparable since a significant degree of selection had been brought into play.

Comparison of the survival periods among the patients observed and treated between 1917 and 1926 and those seen between 1927 and 1936 showed no noteworthy difference. No improvement in results corresponding to the obviously increased efficiency of radiotherapy in the treatment of carcinoma during the same interval could be discerned.

Surgery

Surgery as a therapeutic measure in malignant lymphoma has been generally interdicted, presumably on the basis that the disease is essentially systemic.[56] In this connection it was noted that among the 135 autopsied cases in this series, 10 per cent showed localized lesions, apparently available to surgical excision and certainly not systemic in character. No evidence of the disease was found elsewhere.

In the entire series radical surgery was attempted in 77 cases, generally in ignorance of the histologic character of the lesion. In 44 cases the disease had extended beyond the limits of eradicability at the time of operation. Of the remaining 33 patients, 10 died as the immediate result of the surgical procedure. Twenty-three survivors, 12 of whom were among those listed above as exhibiting unexpectedly prolonged courses, lived or are living, an average of 7.0 years after the onset of their disease. Ten have survived an average of 6.6 years after operation without evidence of recurrence and the remaining 13 continued for an average of 5.7 years postoperatively before any evidence of recurrence appeared. Of those surviving without recurrence, one died of other causes 4.5 years after operation and at necropsy no evidence of residual tumor was found.

The number of individuals receiving radical surgical treatment was admittedly too small and the operative mortality obviously too high to allow unqualified recommendation of such a therapeutic measure. The selection of cases, however, was quite limited and the surgical approach in many was unduly delayed. Considering all of these factors we believe that among the malignant lymphomas there are certain cases with evidence of a single localized lesion, generally, though not always, affecting a bone or a viscus, which may be more successfully treated by radical surgery than by other means.

Summary and Conclusions

A cytologic classification of the malignant lymphomas has been presented and its advantages pointed out in a clinico-pathologic survey of 545 cases (73 cases of the 618 studied histologically had inadequate clinical data). It has been shown, by multiple examinations at significant time intervals, that the cytologic type is remarkably constant, although a few cases show a progressive failure of differentiation as the disease progresses. In contrast, in a classification based largely on distribution, such features as the presence or absence of leukemia, generalization versus localization and "sarcomatous" growth are considered important. These have been shown to be inconstant and changeable, thereby requiring variation in classification from time to time in order to fit the stage of the disease.

It was found that the vast majority of the 618 cases from which histologic material was available could be readily divided into the following seven categories: stem cell lymphoma, clasmatocytic lymphoma, lymphoblastic lymphoma, lymphocytic lymphoma, Hodgkin's lymphoma, Hodgkin's sarcoma and follicular lymphoma. This differs from widely accepted classifications primarily in the subdivision into two types of what has generally been grouped under the heading, reticulum cell sarcoma: one in which the cells are highly undifferentiated and resemble lymphoid stem cells, for which we have proposed the name stem cell lymphoma; and a second in which the cells show recognizable features of differentiation in the direction of tissue phagocytes, which we have accordingly termed clasmatocytic lymphoma. It has also proved useful to divide the tumors showing clear evidence of belonging to the lymphocyte series of cells into lymphoblastic and lymphocytic types depending upon whether the immature or mature cells predominate. Hodgkin's disease, too, has appeared divisible into lymphomatous and sarcomatous types. Follicular lymphoma has been shown to be a form of malignant lymphoma and not, as has been claimed, merely an inflammatory process.

In Part II the value of this classification has been put to the test of

clinical correlation and, although considerable overlapping was observed as would be expected in so closely related a group of diseases, sufficiently constant differences were found in the age of onset, duration of the disease, maximal frequency of involvement of various organs and tissues, tendency to localization or generalization, the development of leukemia and the degree of radiosensitivity to delineate a series of recognizably different clinical syndromes.

The following conclusions appear justified on the basis of the recorded clinical and pathologic observations:

Prognostic implications may be guided to a surprising degree by the histologic character of the lesion. However, unexpectedly prolonged survival periods have been encountered, on the one hand, in cases in which the initial lesion appeared in the skin, bone or viscera and, on the other hand, in those in which the primary lesion was sufficiently circumscribed to be susceptible to surgical extirpation. Five-year survivals have been encountered in all groups but have ranged from 3 per cent of patients with lymphoblastic lymphoma to 53 per cent of those with follicular lymphoma.

As a general rule small doses of roentgen irradiation have a favorable effect upon patients with malignant lymphoma and there is evidence to show that such therapy does prolong life. Patients with clasmatocytic or stem cell lymphoma require somewhat higher dosages to produce beneficial effects and in a few instances, notably in lymphoblastic lymphoma and Hodgkin's sarcoma, irradiation fails to produce any improvement at all.

The presence or development of leukemia cannot be predicted on the basis of any constant morphologic criterion. In fact, very often the blood picture itself is inconstant and varies from time to time during the course of the disease. It seems more reasonable to consider lymphatic leukemia simply as a manifestation of an underlying lymphomatous process. Along the same lines, in the interests of clarity, it would seem judicious to discard such terms as "lymphosarcoma" (Kundrat) and "leukosarcoma" since these, too, appear merely to represent transient phases of malignant lymphoma and do not constitute disease entities.

REFERENCES

1. Callender, G. R. Tumors and tumor-like conditions of the lymphocyte, the myelocyte, the erythrocyte and the reticulum cell. *Am. J. Path.*, 1934, **10**, 443–465.
2. Warthin, A. S. The genetic neoplastic relationships of Hodgkin's disease, aleukaemic and leukaemic lymphoblastoma, and mycosis fungoides. *Ann. Surg.*, 1931, **93**, 153–161.

3. Banti, G. Leukemia and sarcomatosis. *Internat. Clin.*, 1906, 16 s., **3**, 286–298.
4. Helly, Konrad. Leukämien. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1927, **1**, pt. 2, 1028–1030.
5. Rhoads, C. P., and Miller, D. K. Histology of the bone marrow in aplastic anemia. *Arch. Path.*, 1938, **26**, 648–663.
6. Thompson, W. P.; Richter, M. N., and Edsall, K. S. An analysis of so-called aplastic anemia. *Am. J. M. Sc.*, 1934, **187**, 77–88.
7. Sternberg, Carl. Über eine eigenartige unter dem Bilde der Pseudoleukämie verlaufende Tuberkulose des lymphatischen Apparates. *Ztschr. f. Heilk.*, 1898, **19**, 21–90.
8. Reed, D. M. On the pathological changes in Hodgkin's disease, with especial reference to its relation to tuberculosis. *Johns Hopkins Hosp. Rep.*, 1902, **10**, 133–196.
9. Roulet, Frédéric. Das primäre Retothelsarkom der Lymphknoten. *Virchows Arch. f. path. Anat.*, 1930, **277**, 15–47.
10. Roulet, Frédéric. Weitere Beiträge zur Kenntnis des Retothelsarkoms der Lymphknoten und anderer Lymphoiden-Organe. *Virchows Arch. f. path. Anat.*, 1932, **286**, 702–732.
11. Ewing, James. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1928, ed. 3.
12. Klemperer, Paul. The Relationship of the Reticulum to Diseases of the Hematopoietic System. In: Libman Anniversary Volumes. The International Press, New York, 1932, **2**, 665.
13. Medlar, E. M. An interpretation of the nature of Hodgkin's disease. *Am. J. Path.*, 1931, **7**, 499–513.
14. Rhoads, C. P. The reticulo-endothelial system—a general review. *New England J. Med.*, 1928, **198**, 76–78.
15. Parker, Frederic, Jr., and Jackson, Henry, Jr. Primary reticulum cell sarcoma of bone. *Surg., Gynec. & Obst.*, 1939, **68**, 45–53.
16. Selling, Laurence. Benzol as a leucotoxin. Studies on the degeneration and regeneration of the blood and haematopoietic organs. *Johns Hopkins Hosp. Rep.*, 1916, **17**, 83–142.
17. Rosenthal, Maurice, and Grace, E. J. Experimental radium poisoning. I: Bone marrow and lymph-node changes in rabbits, produced by oral administration of radium sulphate. *Am. J. M. Sc.*, 1936, **191**, 607–618.
18. Wiseman, B. K. Lymphopoiesis, lymphatic hyperplasia, and lymphemia: fundamental observations concerning the pathologic physiology and interrelationships of lymphatic leukemia, leukosarcoma and lymphosarcoma. *Ann. Int. Med.*, 1935–36, **9**, 1303–1329.
19. Meyer, Erich, and Heineke, Albert. Ueber Blutbildung in Milz und Leber bei schweren Anämien. *Verhandl. d. deutsch. path. Gesellsch.*, 1905, **9**, 224–228.
20. Pappenheim, Artur. Atlas der menschlichen Blutzellen. Gustav Fischer, Jena, 1912.
21. Farrar, G. E., Jr., and Cameron, J. D. Monocytic leukemia with data on the individuality and development of the monocyte. *Am. J. M. Sc.*, 1932, **184**, 763–770.
22. Ferrata, Adolfo. Le Emopatie. Soc. Ed Libr., Milano, 1918, 1 (parte générale).
23. Turnbull, H. M. The Anatomy of Erythropoiesis. In: Vaughan, J. M. The Anemias. Oxford University Press, London, 1936, ed. 2, p. 11.
24. Ehrlich, J. C., and Gerber, I. E. The histogenesis of lymphosarcomatosis. *Am. J. Cancer*, 1935, **24**, 1–35.

25. Richter, M. N. Generalized reticular cell sarcoma of lymph nodes associated with lymphatic leukemia. *Am. J. Path.*, 1928, **4**, 285–292.
26. Robb-Smith, A. H. T. Reticulosis and reticulosarcoma: a histological classification. *J. Path. & Bact.*, 1938, **47**, 457–480.
27. Maximow, A. A., and Bloom, William. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1935, ed. 2.
28. Doan, C. A. The type of phagocytic cell and its relative proportions in human bone marrow and spleen, as identified by the supravital technique, with special reference to pernicious anemia. *J. Exper. Med.*, 1926, **43**, 289–296.
29. Sabin, F. R. On the origin of the cells of the blood. *Physiol. Rev.*, 1922, **2**, 38–69.
30. Sabin, F. R., and Doan, C. A. The relation of monocytes and clasmatocytes to early infection in rabbits with bovine tubercle bacilli. *J. Exper. Med.*, 1927, **46**, 627–644.
31. Osgood, E. E., and Ashworth, C. M. Atlas of Hematology. J. W. Stacey, Inc., San Francisco, 1937.
32. Sabin, F. R. Cellular reactions to tuberculo-proteins compared with the reactions to tuberculo-lipids. *J. Exper. Med.*, 1938, **68**, 837–852.
33. Edling, L. Contribution to the pathology and clinical picture of reticulum-cell sarcoma. *Radiology*, 1938, **30**, 19–34.
34. Kundrat. Ueber Lympho-Sarkomatosis. *Wien. klin. Wchnschr.*, 1893, **6**, 211–213; 234–239.
35. Jackson, Henry, Jr. The classification and prognosis of Hodgkin's disease and allied disorders. *Surg., Gynec. & Obst.*, 1937, **64**, 465–467.
36. Jackson, Henry, Jr. Hodgkin's disease and allied disorders. *New England J. Med.*, 1939, **220**, 26–30.
37. Smith, C. A. Hodgkin's disease in childhood. A clinical study with a résumé of the literature to date. *J. Pediat.*, 1934, **4**, 12–38.
38. Mueller, Theodor. Relation of Hodgkin's disease to sarcoma. With a report of two cases. *J. M. Research*, 1920–21, **42**, 325–338.
39. Brill, N. E.; Baehr, George, and Rosenthal, Nathan. Generalized giant lymph follicle hyperplasia of the lymph nodes and spleen. A hitherto undescribed type. *J. A. M. A.*, 1925, **84**, 668–671.
40. Baehr, George, and Rosenthal, Nathan. Malignant lymph follicle hyperplasia of spleen and lymph nodes. (Abstract.) *Am. J. Path.*, 1927, **3**, 550.
41. Baehr, George. The clinical and pathological picture of follicular lymphoblastoma. *Tr. A. Am. Physicians*, 1932, **47**, 330–338.
42. Symmers, Douglas. Follicular lymphadenopathy with splenomegaly. A newly recognized disease of the lymphatic system. *Arch. Path.*, 1927, **3**, 816–820.
43. Symmers, Douglas. Giant follicular lymphadenopathy with or without splenomegaly. Its transformation into polymorphous cell sarcoma of the lymph follicles and its association with Hodgkin's disease, lymphatic leukemia and an apparently unique disease of the lymph nodes and spleen—a disease entity believed heretofore undescribed. *Arch. Path.*, 1938, **26**, 603–647.
44. Gall, E. A.; Morrison, H. R., and Scott, A. T. The follicular type of malignant lymphoma; a survey of 63 cases. *Ann. Int. Med.*, 1941, **14**, 2073–2090.
45. Forkner, C. E. Leukemia and Allied Disorders. The Macmillan Co., New York, 1938.
46. Gibbons, H. W. The relation of Hodgkin's disease to lymphosarcoma. *Am. J. M. Sc.*, 1906, **132**, 692–704.
47. Flashman, D. H., and Leopold, S. S. Leukosarcoma. With report of a case beginning with a primary retroperitoneal lymphosarcoma and terminating with leukemia. *Am. J. M. Sc.*, 1929, **177**, 651–663.

48. Baldridge, C. W., and Awe, C. D. Lymphoma. A study of 150 cases. *Arch. Int. Med.*, 1930, **45**, 161–190.
49. Brunschwig, Alexander, and Kandel, Ernestine. A correlation of the histologic changes and clinical symptoms in irradiated Hodgkin's disease and lymphoblastoma of lymph nodes. *Radiology*, 1934, **23**, 315–326.
50. Sternberg, Carl. Ueber Leukosarkomatose. *Wien. klin. Wchnschr.*, 1908, **21**, 475–480.
51. Case records of the Massachusetts General Hospital, case no. 23031. *New England J. Med.*, 1937, **216**, 116–118.
52. Farley, D. L. A review of the treatment of the lymphatic leukemias and related diseases especially by irradiation. *Medicine*, 1928, **7**, 65–103.
53. Desjardins, A. U. Radiotherapy for Hodgkin's disease and lymphosarcoma. *J. A. M. A.*, 1932, **99**, 1231–1236.
54. Minot, G. R., and Isaacs, Raphael. Lymphoblastoma (malignant lymphoma). Age and sex incidence, duration of disease, and the effect of roentgen-ray and radium irradiation and surgery. *J. A. M. A.*, 1926, **86**, 1185–1189; 1265–1270.
55. Minot, G. R. Lymphoblastoma. *Radiology*, 1926, **7**, 119–120.
56. Singer, H. A. Primary, isolated lymphogranulomatosis of the stomach. *Arch. Surg.*, 1931, **22**, 1001–1017.

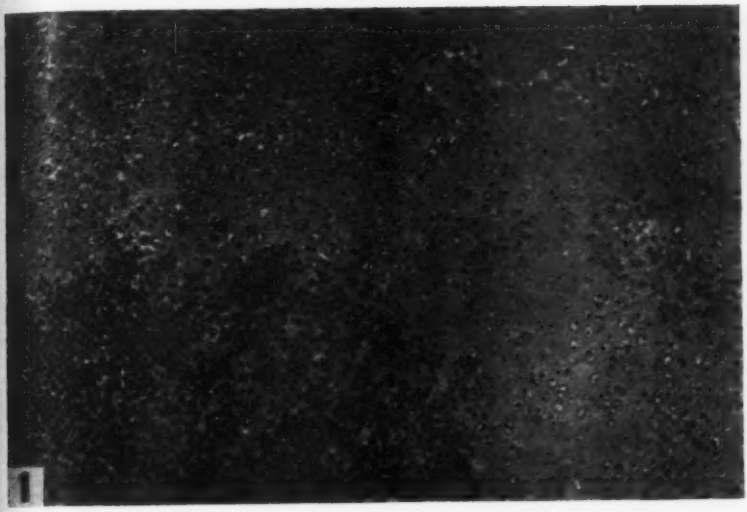
DESCRIPTION OF PLATES

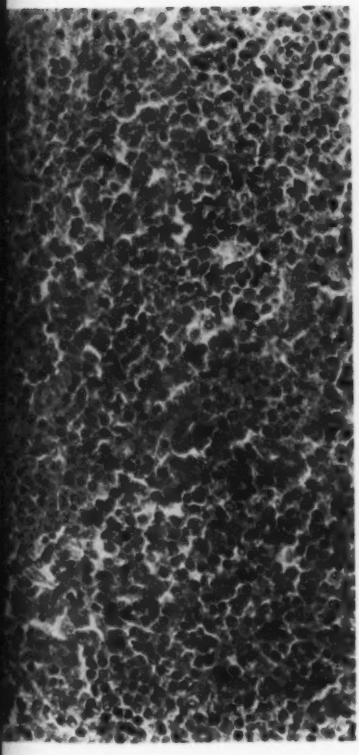
PLATE 62

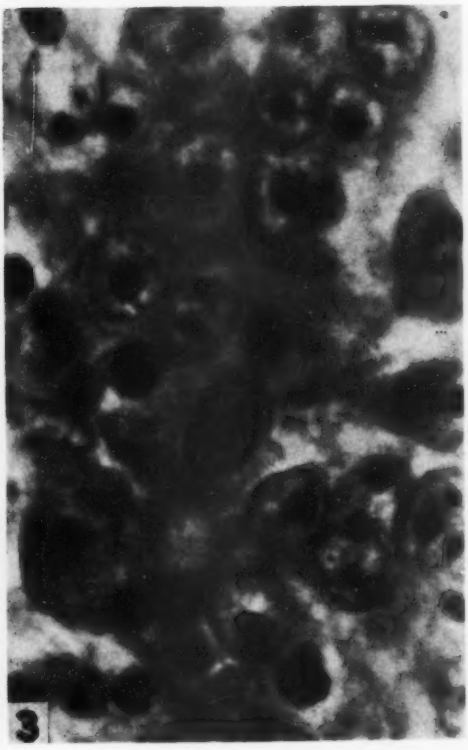
FIG. 1. (Lymph node). Stem cell lymphoma, syncytial type. The dark staining area in the left lower corner of the photomicrograph represents residual normal lymphocytes in the process of being displaced by invading tumor. The infiltrating syncytial mass produces the lighter staining region in which large, clear nuclei with prominent nucleoli may be seen. Cell boundaries cannot be distinguished. × 200.

FIG. 2. (Lymph node). Stem cell lymphoma in which the cells have become discrete and intercellular connections minimized. Nuclear characteristics are unchanged from those noted in the syncytial type. × 200.

FIG. 3. (Lymph node). Stem cell lymphoma, syncytial type. The nuclei are large, clear and contain prominent, densely staining nucleoli. All cytoplasmic substance is fused and there are no cell boundaries apparent. × 1000.







and Mallory

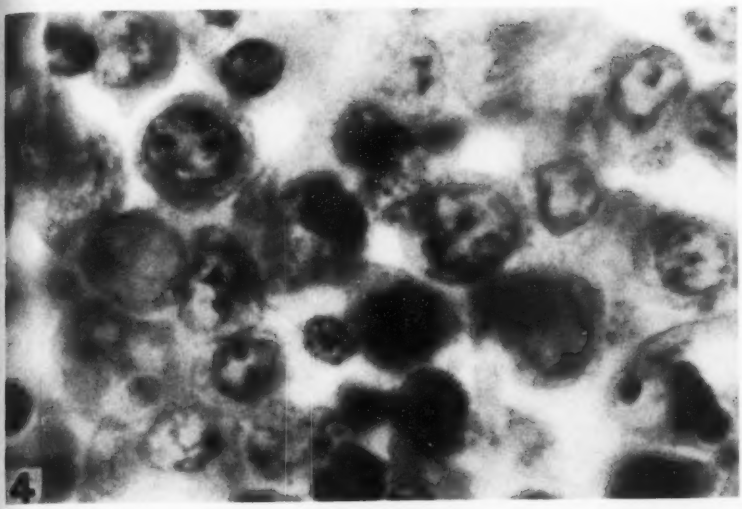
Malignant Lymphoma

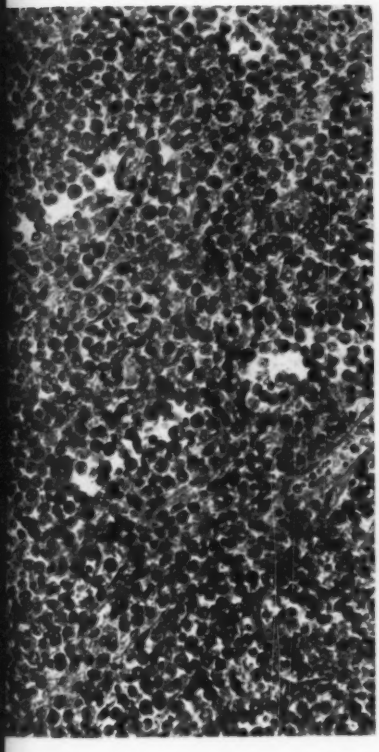
PLATE 63

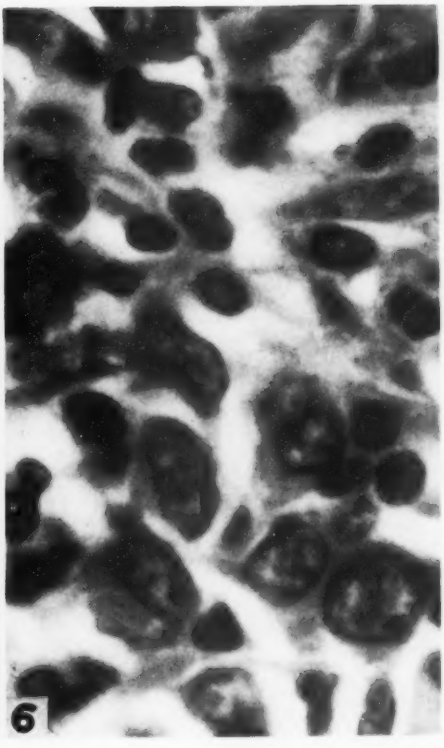
FIG. 4. (Lymph node). Stem cell lymphoma. Nuclear characteristics are essentially similar to those noted in Figure 3. Although intercellular connections may be seen, cellular identity is now evident. There are a few dark staining lymphocytes present in this section. × 1000.

FIG. 5. (Lymph node). Clasmatocytic lymphoma. Cells similar in size to those noted in stem cell lymphoma comprise this lesion. Cytoplasm is abundant, distinctly outlined, and nuclei exhibit a marked degree of variation in configuration. × 200.

FIG. 6. (Lymph node). Clasmatocytic lymphoma. Characteristic cells show an abundant, clearly delimited cytoplasm which occasionally contains small vacuoles. Nuclei are eccentric, contain a reticulated chromatin and are reniform in shape. A stem cell is present in the right lower corner and there are several lymphocytes scattered throughout the field. × 1000.







nd Mallory

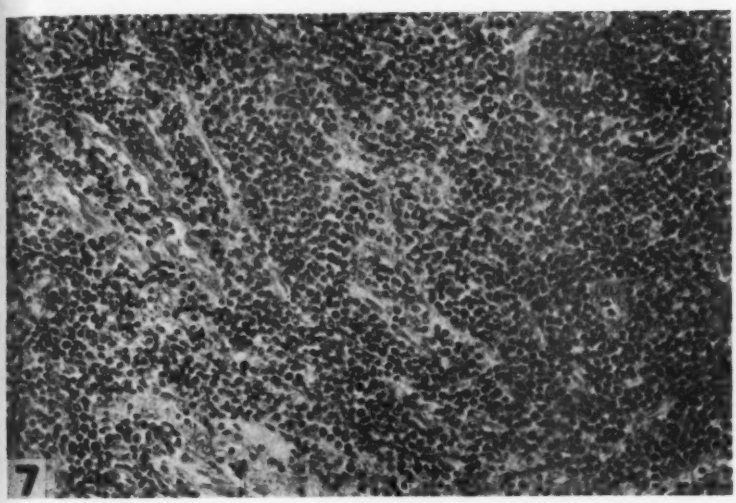
Malignant Lymphoma

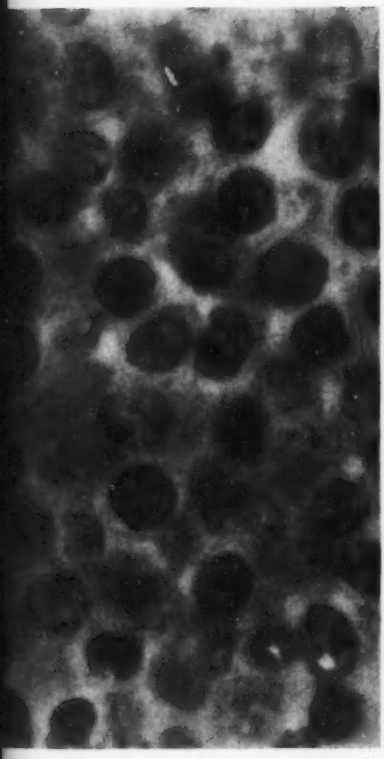
PLATE 64

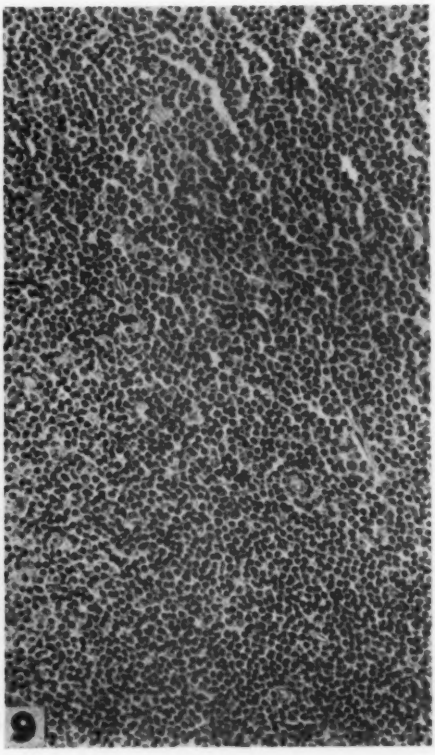
FIG. 7. (Lymph node). Lymphoblastic lymphoma with revision of nodal architecture by closely packed lymphoblasts. × 200.

FIG. 8. (Lymph node). Lymphoblastic lymphoma; predominant cells exhibit scanty cytoplasm with larger and paler nuclei than those observed in the lymphocytic type. Chromatin is less prominent and more diffusely distributed. × 1000.

FIG. 9. (Lymph node). Lymphocytic lymphoma with replacement of normal nodal structure by uniformly distributed, small lymphocytes. × 200.







nd Mallory

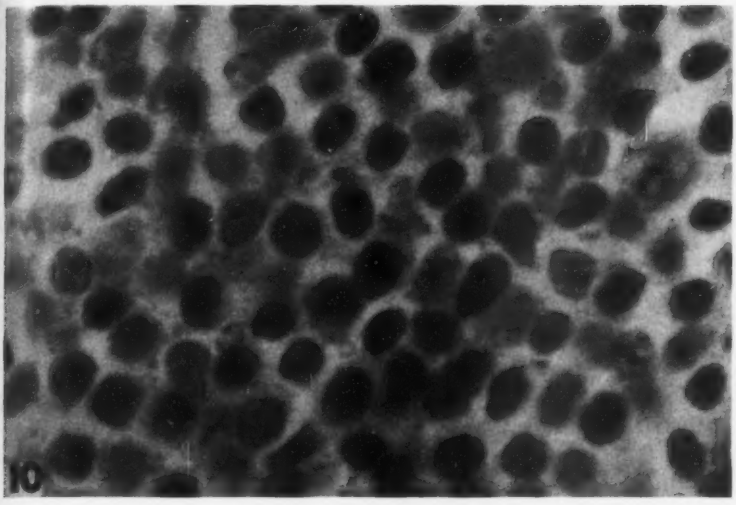
Malignant Lymphoma

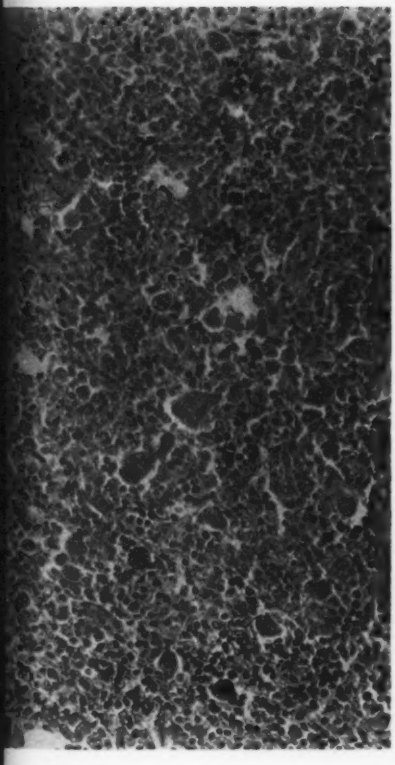
PLATE 65

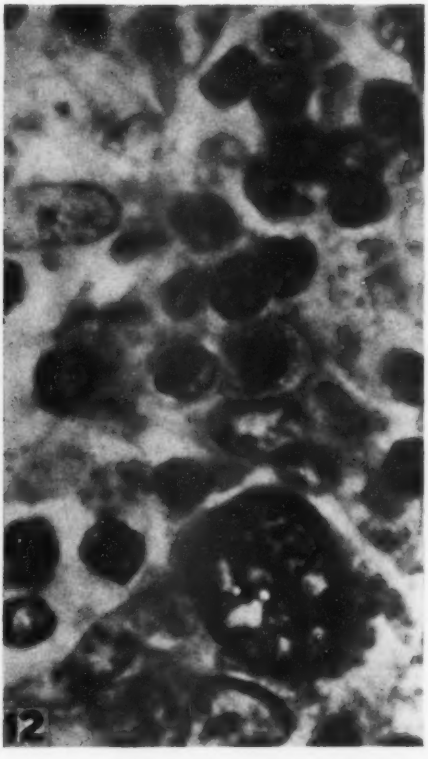
FIG. 10. (Lymph node). Lymphocytic lymphoma showing predominance of small lymphocytes with dark staining, heavily chromatinized nuclei. An occasional, large, pale nucleus of a less mature cell may be seen. × 1000.

FIG. 11. (Lymph node). Hodgkin's lymphoma. Characteristic multinucleated giant cells are obvious. There is also a marked degree of polycellularity. × 200.

FIG. 12. (Lymph node). Hodgkin's lymphoma. In the lower portion of the photomicrograph there is a Sternberg-Reed giant cell. Scattered throughout the area may be seen lymphocytes, monocytes, fibroblasts and an occasional granulocyte. × 1000.







and Mallory

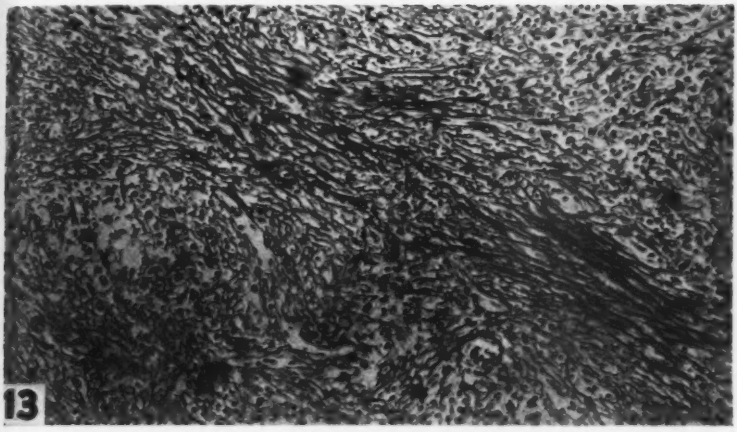
Malignant Lymphoma

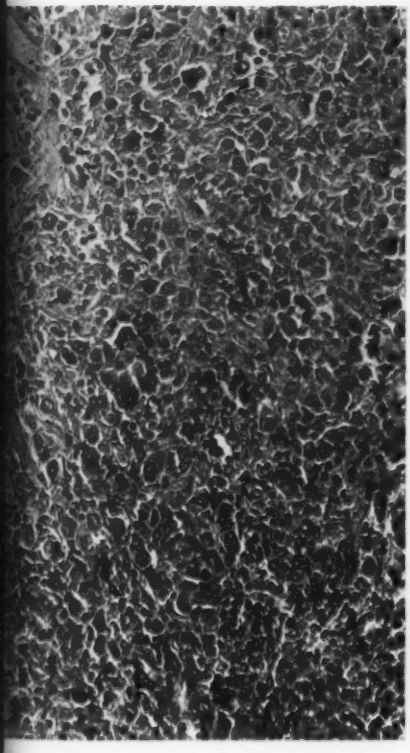
Plate 66

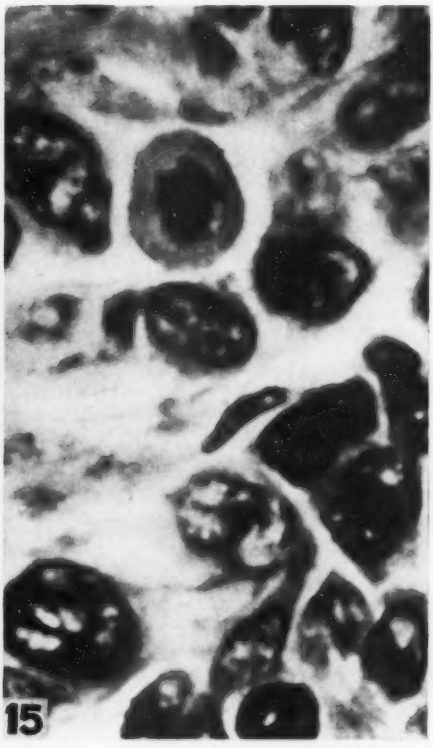
Fig. 13. (Lymph node). Hodgkin's lymphoma, scirrhous type. Broad strands of fibrous tissue infiltrate among the tumor cells, producing islet arrangement. × 150.

Fig. 14. (Lymph node). Hodgkin's sarcoma. The lesion consists almost wholly of Sternberg-Reed cells with a background of fibrous tissue. Relatively few mature cells are present. × 200.

Fig. 15. (Lymph node). Hodgkin's sarcoma. Giant cells with bizarre, irregular and multiple nuclei are apparent. The only other recognizable elements are fibroblasts. × 1000.







and Mallory

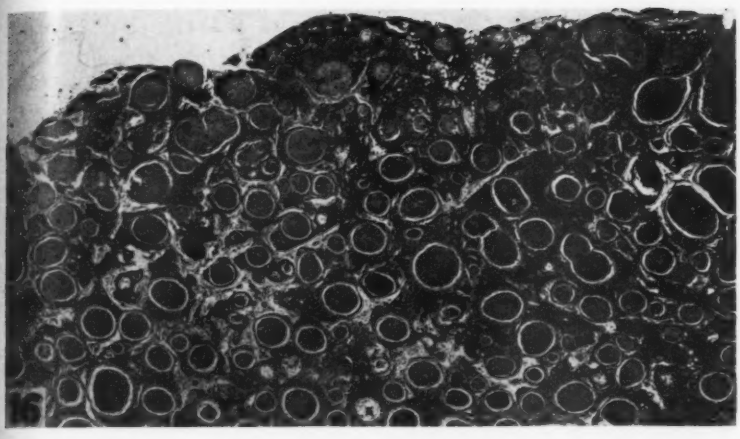
Malignant Lymphoma

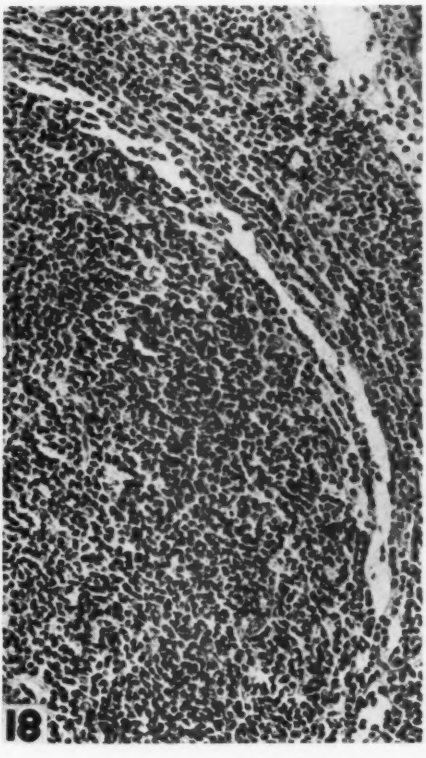
PLATE 67

FIG. 16. (Lymph node). Follicular lymphoma. Normal architecture is revised by considerably increased numbers of follicles which vary considerably in size. Clear spaces surrounding each one represent the "cracking off" phenomenon described in the text. × 50.

FIG. 17. (Spleen). Follicular lymphoma. Follicles are enormous and extremely irregular in configuration. Fusion of adjacent follicles may be seen. × 50.

FIG. 18. (Lymph node). Follicular lymphoma. Similarity of cells (small lymphocytes) in the pulp and follicles may be seen. The "cracking off" phenomenon is evident. × 300.





and Mallory

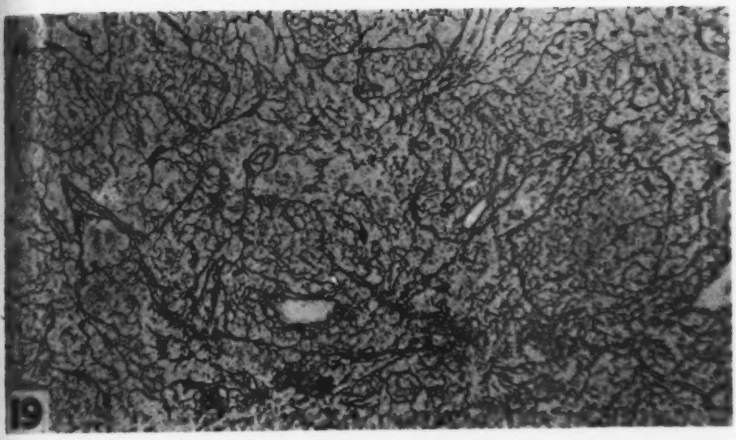
Malignant Lymphoma

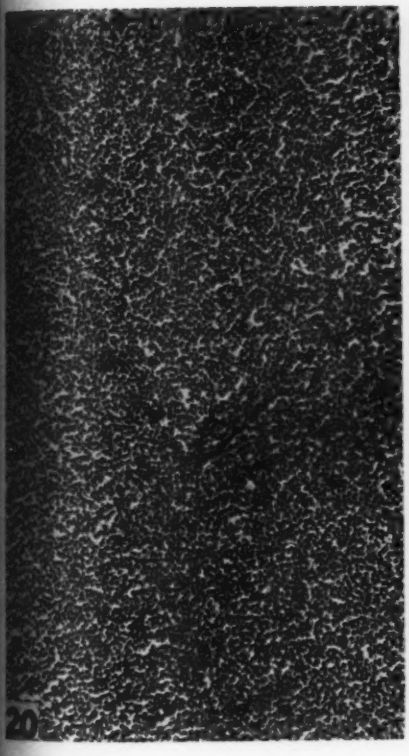
PLATE 68

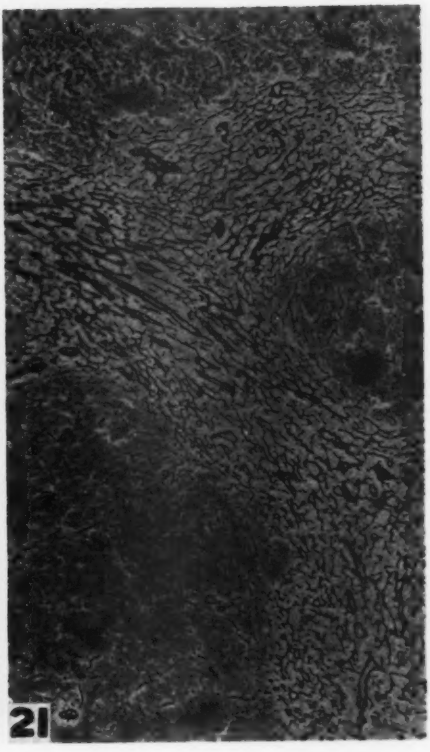
FIG. 19. Normal lymph node (Perdrau silver stain). The fine fibrillar framework is well shown and there is condensation at the edge of the sinuses. Although present in both follicles and sinuses, reticulum is scanty in these regions. × 200.

FIG. 20. Lymphocytic lymphoma (Perdrau silver stain). The normal meshwork has been replaced and only a few, fine argentophilic fibrils are seen. × 200.

FIG. 21. Follicular lymphoma (Perdrau silver stain). Stromal reticulum is obviously compressed and interfibrillar spaces narrowed and elongated. Sinuses are not seen. Reticulum content of follicles is remarkably scanty. × 200.







Gall and Mallory

Malignant Lymphoma

THE FIBROUS CONNECTIVE TISSUE OF THE ARTIFICIALLY INDUCED MATERNAL PLACENTA IN THE RAT WITH PARTICULAR REFERENCE TO THE RELATIONSHIP BETWEEN RETICULUM AND COLLAGEN *

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Previously we [1,2] have described changes associated with advancing age in the connective tissue of the uterus of the rat. It was ascertained that beginning early in life (approximately 10 days) there was a transformation of reticulum into collagen, which was largely completed in the endometrium by 90 days of age. However, throughout life, there was a progressive deposition of additional collagenous connective tissue. Thus, for rats of different ages, it was found that the uterine connective tissue, particularly in the endometrium, assumed fairly characteristic patterns.

In this study we were impressed by one constant finding: stromal cells in the endometrium were much more abundant in those areas where reticulum was found than in those in which the fibers were collagenous. Since it is generally agreed that connective tissue cells play a dominant rôle in the origin of fibers and since it is quite likely that these cells exert a definite effect on the fibers throughout life, it was considered of some interest to ascertain what would happen to the endometrial connective tissue fibers if the structure and activity of the stromal cells were markedly altered. This was easily accomplished in the uterus of the rat by the production of maternal placentae (deciduomata †) by means of irritation. Under such conditions it is well known that the stromal cells undergo marked proliferation and hypertrophy to form decidual cells. Our purpose, therefore, was to study the fibrillar material found in deciduomata and to correlate the changes which occur in the endometrial connective tissue fibers with those which occur in the stromal cells.

Since the findings reported in this paper have a bearing on the general problem of the relationship between reticulum and collagen, several facts pertinent to this subject should be considered. In 1891, Mall [3] described fine connective tissue fibrils in many organs which branched

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† Although the term deciduoma implies a uterine neoplasm made up of decidual cells, it is also used to denote the mass of decidual cells (maternal placenta) that can be made to appear in the uteri of laboratory animals by artificially stimulating the endometrium when the uterus is under the influence of the hormone of the corpus luteum. The term is used in this sense in this paper.

and formed anastomosing networks. He called these fibrils reticulum and differentiated them from collagen by means of chemical and digestive experiments.[4] He considered them identical to the fine fibrils (Gitterfasern) of the liver which had been described by Kupffer,[5] in 1876, and which Oppel,[6] in 1890, had reported could be impregnated by his silver chromate method. In 1905, Maresch [7] modified the Bielschowsky silver technic for use on non-nervous tissue and in the next few years it was applied to many organs by numerous investigators.[8,9] It was found that following this procedure the fine connective tissue fibrils stained black whereas the characteristic collagenous bundles stained old rose or reddish (yellow or golden if the gold chloride bath was omitted). It has been generally assumed that the fibers which Mall has designated as reticulum are identical with the argyrophilic fibers. Furthermore, this staining reaction has come to be generally accepted as a method of differentiating reticular from collagenous fibers.

There has been much discussion concerning the ultimate relationship between the two morphologic types of connective tissue and at the present time there are two schools of thought concerning this problem. Mallory and Parker [10] and others have presented evidence indicating that the only difference between these fibers is physical. According to this view, the fine fibrils are easily impregnated and therefore are argyrophilic, while the larger bundles are not affected because of their thickness. On the other hand, Foot [11] supports the original views of Mall and thinks that the difference in staining reaction is due to chemical differences in the fibers. Regardless of the exact nature of this difference, it seems generally accepted that reticulum is a precollagenous type of tissue which may be transformed into collagen,[12-14] the fibers in the transitional state staining blackish red or purple. As far as we are aware, no work has been published which would indicate that this transformation is reversible.

Our observations indicate that during the development of deciduomata in rats, a certain amount of the endometrial collagen is transformed into reticulum. Although it was found that the width of a fiber or fibril was of importance in determining its staining reaction, there was also evidence to indicate that the location of the fiber and possibly the structure and metabolic activity of the surrounding cellular elements might play a definite rôle in determining whether or not a fiber will impregnate with silver.

Materials and Methods

Twenty-three virgin female rats were used. Pseudopregnancy was induced by mating with a vasectomized male. Vaginal smears were made daily prior to the mating and throughout the experimental period.

On the fifth day of pseudopregnancy a uterine biopsy was obtained from the right horn of the uterus (deciduomata were usually found later at the cut surfaces). Deciduomata were stimulated in the left horn at two sites by passing threads through the uterus and at one site by making a longitudinal slit, approximately 5 mm. in length. Animals were killed at 3, 5, 8 and 10 days after uterine irritation (8th, 10th, 13th and 15th days of pseudopregnancy, respectively). A total of 102 deciduomata were found and studied. Those induced by slitting were generally the largest while those at the site of biopsies were the smallest; those induced by threading were intermediate. The pattern of the decidual changes occurring in the latter most nearly resembled those found in deciduomata which were induced without mechanical injury.[15] Therefore, our description is based largely on this type.

At autopsy, approximately half of the deciduomata were fixed in a 10 per cent solution of neutral formaldehyde, the remainder in Bouin's fluid. Interrupted serial sections were made from each deciduoma. Sections from those fixed in formaldehyde were so arranged that alternate ribbons of five sections were placed on separate sets of slides. One set of these was stained by Mallory's triple stain, while the second was stained by Gömöri's [16] modification of the Bielschowsky-Maresch silver impregnation method for the differentiation of reticulum and collagen. By the method of mounting, consecutive sections, one stained by the silver method and the other by Mallory's stain, could be compared. The Bouin-fixed tissue was stained only by Mallory's method. Normal uterine tissue obtained at biopsy and autopsy was fixed in both fixatives and stained by both methods.

The Gömöri [16] technic stains the reticulum black and collagen rose-red. Fibers transitional between reticulum and collagen stain purplish or reddish black. This differential staining reaction is used as the sole basis for the classification of fibrillar material. The term "argyrophilic" will be used interchangeably with "reticular." Nuclei usually stain reddish brown but in some instances are grayish. Cytoplasm is usually reddish brown but sometimes dark gray or almost black. The staining reactions of Mallory's stain are well known.

In order to study the changes in the endometrial connective tissue which occurred at the sites of deciduomata, it was considered necessary to know fairly exactly the condition of the endometrium at the time of irritation. All animals were within 2 or 3 weeks of 1 year of age at the time of autopsy. From our previous studies, the condition of the endometrium of rats of this age was well known. This control material was supplemented by the uterine biopsies, obtained at the time of irritation, which made it possible to study, in the case of each rat, a sample of the very endometrium in which the deciduomata subsequently developed.

Observations

The normal endometrial connective tissue of rats of this age was found to be entirely collagenous (Figs. 21 and 23), with the exception of a reticular basement membrane, a narrow zone containing a meshwork of reticulum (subepithelial reticular zone) immediately underlying and continuous with the basement membrane (Fig. 21), and a few thin, reticular fibrils at the bases of gland cells and around blood vessels (Fig. 23). Thus, the collagenous zone of the endometrium extended from the inner border of the circular smooth muscle almost to the lumen and comprised about 80 to 90 per cent of its thickness. The collagen was arranged either as a dense meshwork of fibers, 2 to 4 μ in width, or as thicker fibers, up to 10 μ, concentrically arranged around the glands (Figs. 23 and 24) or lying in fairly regular rows more or less parallel to the circular smooth muscle (Fig. 23). The fibers were thickest and most densely arranged deep in the endometrium (Fig. 23). As the lumen was approached, thinner fibers arranged in a meshwork were encountered and at the lower limits of the subepithelial zone they blended almost imperceptibly with the reticular fibrils (Fig. 21).

Stromal cells of the endometrium were definitely most abundant in the subepithelial zone of reticulum (Fig. 22). In the collagenous portion they were less numerous and in the deeper parts, where the collagenous fibers were wider and more densely arranged, they were so few as to be inconspicuous. The cells lying between the thicker fibers were often compressed and spindle-shaped.

Structural details of deciduomata in rats have been described by Selye and McKeown [17] and Krehbeil.[15] They have shown these growths to be composed of two general types of decidual cells: small glycogen-containing cells located at the mesometrial pole, and larger cells which contain lipoid material located at the antimesometrial pole. We have found both of these types with intermediate variants between them. In regressing deciduomata, Selye and McKeown also described large granular cells in the mesometrium, forming a structure they called the "metrial gland," a finding we have confirmed. Although the present paper is concerned chiefly with fibrous tissue, we have studied the more general histologic details of deciduomata, including growth and degeneration. Our general observations largely confirm and in some instances supplement those of the writers cited.

It is known that deciduomata reach the peak of their development about 5 days after uterine irritation (tenth day of pseudopregnancy). These we are describing in detail in order to establish the normal pattern of the fibrillar material in the fully developed deciduoma. Less

detailed descriptions will be given of deciduomata in rats killed 3 days following uterine irritation in which the growths were still in a developmental state, and in rats killed 8 days or more after uterine irritation in which the deciduomata, including the fibrillar material, were degenerating.

Deciduomata from Rats 5 Days After Uterine Irritation (Tenth Day of Pseudopregnancy)

The 43 deciduomata (Fig. 39) studied were composed of two general types of decidual cells: small cells (Fig. 25) generally located mesometrially and large cells (Fig. 26) most often situated at the antimesometrial pole. Transitional types were also found (Fig. 1); often cells of the same general type differed in size and appearance. Near the center of the decidual mass there was usually a small cavity or cleft containing blood (Fig. 39). This space, sometimes lined with low epithelium, represented a portion of the lumen which had been pinched off from the remainder of the cavity by the rapid growth of the decidual cells.

At the mesometrial pole the small decidual cells generally extended to the inner border of the circular smooth muscle (Fig. 39), although in some of the smaller growths a thin layer of endometrium next to the muscle layer persisted. Antimesometrially, a thicker layer of normal endometrium was usually found (Fig. 39) and this increased in thickness laterally from the center of the deciduoma toward the normal uterus on each side. The amount of the persisting endometrium at the two poles was apparently determined by the size of the decidual mass and the amount of endometrium primarily involved.

The structure of the small decidual cells was best observed in the tissue fixed in Bouin's fluid and stained by Mallory's method. Those well within the decidual mass usually differed somewhat in appearance from those near the periphery. The former were generally stellate in shape with numerous fine processes, many of which were in contact with those of neighboring cells (Fig. 25); the exact structural relationship between the processes of adjoining cells was not ascertained. Relatively wide intercellular spaces were found between the cells (Fig. 25) and their cytoplasm usually stained reddish brown and was generally, but not always, scanty. The cytoplasm was finely reticulated and often fine red granules were seen. A distinct cell membrane could not be observed. Most cells contained one nucleus, but in some two were seen; one or more prominent nucleoli were observed.

In most instances it could be demonstrated that the cells, and apparently their processes, were closely surrounded by a thin layer of blue intercellular material. In these preparations it could not be defi-

nitely determined whether or not this fine intercellular material was actually fibrillar. Thin strands of this substance extended out into the intercellular spaces and formed a meshwork. The intercellular spaces appeared irregularly rounded and were bounded both by cell processes and strands of intercellular material (Fig. 25). We feel that they are the sites of accumulation of fluid lying in the intercellular material. A few definite connective tissue fibers were seen; they were relatively wide, measuring up to 3 μ, and usually wavy. As will be seen later, they were actually segments of longer fibers. They stained more deeply than the finer intercellular material, but had no demonstrable spatial relationship to the cells. Since in the Mallory preparations they did not come into good focus at the same point as the decidual cells, it was difficult to demonstrate them in photomicrographs of these sections.

Near the periphery of the deciduoma, the small decidual cells were generally more compactly arranged, the cell processes being less abundant, but shorter and thicker and often appearing continuous with those of adjoining cells. The intercellular spaces were less numerous, but generally large and irregularly rounded. Fine strands of intercellular material, similar to that described above, closely surrounded the cells and the intercellular spaces.

In the silver (Gömöri) preparations, a much clearer concept of the nature of the intercellular material among the small decidual cells could be gained (Figs. 27 to 32). The fibrillar elements, which in most instances were quite wavy, ranged from very fine fibrils to fibers up to 3 μ in thickness (Figs. 2 to 14 and 27 to 32). All but the very finest often showed a cut surface at each end which was slightly expanded (Figs. 4, 7 and 8). Generally speaking, fibrillar material was more abundant at the periphery of the decidual mass where it blended with that of the adjoining endometrium (Figs. 35 and 36).

The fine fibrils were almost invariably argyrophilic and were therefore considered to be reticulum (Figs. 2, 3, 11 and 31). In some instances the thick fibers (2 or 3 μ in thickness) were also argyrophilic (Figs. 9, 10, 14 and 28); in others they were either collagenous (Fig. 13) or transitional between reticulum and collagen (Figs. 4, 7, 8 and 29). Thin fibers (approximately 1 μ in diameter) were generally reticular (Fig. 10) or transitional (Figs. 5 and 11). These observations are of considerable interest when it is remembered that in the normal endometrium only the finest fibrils (only a fraction of 1 μ thick as determined by methods not absolutely accurate) were argyrophilic, while thicker elements were invariably collagenous.

Study of the various figures showing silver preparations, and particularly Figures 2 to 14, will reveal a definite structural characteristic of

the fibrillar elements of deciduomata, namely, a marked tendency for the fibers to branch or to become separated into finer elements. This occurred among reticular, transitional and collagenous elements. Often the finer fibrils, partially separated from the thicker fibers, were more argyrophilic than the parent fiber (Figs. 7, 8 and 11). Quite frequently, the lateral borders of the fibers were stained black while the central portion was transitional or collagenous (Figs. 5 and 13). The significance of branching and of the staining reaction will be considered later.

The relationship between the fibrillar material and the small decidual cells can best be understood by studying Figures 27 to 32. Although there was some tendency toward a characteristic arrangement of fibrillar material throughout the entire area occupied by the small decidual cells, there was, nevertheless, a definite variation in the relationship of these elements to the decidual cells. Because of this variation, we will describe several of the most common structural patterns found.

The most typical arrangement is shown in Figure 28. It is seen that in such areas the fine reticular fibrils lined the large and prominent intercellular spaces and were also closely applied to the decidual cells and their processes. The fine argyrophilic "granules" were considered to be fibrils cut transversely. Study of such areas as these strongly indicates that the intercellular material, which in the Mallory preparations was applied to the small decidual cells, was fibrillar. The thicker fibers are well shown in this section. In contrast to the thin fibrils they did not seem to bear any constant relation to the cells. As we have stated before, these thicker fibers were sometimes reticular, but most often they were either transitional or collagenous. This is the type of fiber already referred to which did not photograph well in the Mallory preparations.

Another structural pattern, found almost as frequently as that just described, is shown in Figure 29. The cell processes were fine and apparently anastomosed with those of adjacent cells. The intercellular spaces were small and the number of reticular fibrils found among the cells was much less than in Figure 28. Thicker fibers were also present; they were unusually abundant in this particular section.

An arrangement of the fibrillar elements and decidual cells often found adjoining the remains of the uterine lumen is shown in Figure 27. Here a very regular reticular meshwork was formed about single decidual cells. Intercellular spaces or thicker fibers were most often not present. This type of arrangement was found infrequently in this group but was quite prevalent in the 3-day group.

In Figure 30 is shown the type of structure often found near the

periphery of a deciduoma. The cells were somewhat irregularly arranged and often appeared fused. The intercellular spaces were relatively large and in many places fine reticular fibrils were in contact with the cells and formed a lining for the irregular spaces. As usual the thicker fibers were irregularly arranged in relation to the decidual cells. Structural conditions similar to those shown in Figures 31 and 32 were less often observed near the peripheral portion of decidual growths. The fibrillar elements were variable in size and followed no definite pattern in their relationship to the decidual cells. The branching and the variable form of the fibers are well shown in these two figures.

These observations indicate that there was a tendency for fine reticular fibrils to lie in close contact with the small decidual cells and at the same time to form a lining for the intercellular spaces. However, this was not a constant finding; in some areas there did not seem to be sufficient reticulum present to make such an arrangement possible (Fig. 29), and in other areas there was no constant structural relationship between the cells and reticulum fibrils although the latter were present in large numbers (Fig. 31). In no instance were the thicker fibrillar elements found to bear a constant relationship to the small decidual cells.

The large decidual cells stained by the Mallory method are pictured in Figure 26. They were usually polygonal in shape and closely packed. In general, in those cells which were in contact with each other, cell membranes were visible; sometimes they were not. On the other hand, cell membranes could not be demonstrated in cells which occurred singly, a finding for which we have no explanation. The cytoplasm usually stained reddish brown, appeared finely reticulated and often contained small red granules. The cells were often binucleate with prominent nuclear membranes and large nucleoli. In the silver preparations (Fig. 33) it was found that fibrillar material among these cells was generally much less abundant than among the small decidual cells, although in some instances it was present in considerable amounts among those cells located near the periphery of the decidual mass (Fig. 34). The fibrils were most often thin and reticular although thicker fibers, either transitional or collagenous, were observed. The fibers passed between or over the decidual cells and apparently formed no definite pattern.

Decidual cells which were transitional between the small and large types were found near the junction of the mesometrial and antimesometrial regions (Fig. 39; area marked by arrow). In the Mallory preparations they were of intermediate size and formed a graded series

between the large and small decidual cells. In this transitional area the decidual cells became smaller and less closely packed, and intercellular spaces identical with those among the small decidual cells appeared between them (Fig. 1). Cell processes were generally in contact with those of adjoining cells and often there appeared to be cytoplasmic continuity as well. Abundant light blue intercellular material was in contact with the cells and formed a fine weblike mesh between them and the wide intercellular spaces (Fig. 1). In some instances, irregular masses of fine "granular-appearing" intercellular material, possibly coagulated intercellular fluid, were found between the cells. The intercellular material as it appeared in the Mallory preparations could not definitely be described as fibrillar. In addition to the weblike intercellular substance, a few thick fibers were found. They stained more darkly and were definitely fibrillar. In the silver sections, the intercellular material between these transitional cells had a different appearance. Fine fibrils surrounded the cells and reproduced to a certain degree the fine meshwork observed in the Mallory preparations. No argyrophilic material resembling the "granular-appearing" substance mentioned above was noticed. The thick fibers were prominent.

The structure at the junction of the decidual growth and the normal endometrium varied, two main types being found (Figs. 20, 35 and 36). In the first, only a narrow transitional zone appeared between the decidual growth and the decidual endometrium and the separation between the two types of tissue was fairly sharp and distinct. In this type there was a tendency for the more peripheral decidual cells to be compressed. Outside of these, there was often a narrow zone which blended with the adjoining endometrium in which the stromal cells were slightly hypertrophied.

The second and more common type of junction was much less clear-cut and extended throughout a relatively wide transitional zone (Figs. 20, 35 and 36). In this zone the cells showed moderate hypertrophy, which was greatest immediately adjoining the decidual growth and became progressively less in the adjacent endometrium. The fibrillar material presented comparable changes. The broad and dense collagenous fibers of the adjoining endometrium lost their regular pattern and many became split up into finer elements to form an irregular meshwork (Figs. 35 and 36) about the moderately altered cells. The finer elements were either reticular or transitional in nature; the coarser were collagenous. In many instances broad collagenous fibers extended into the peripheral portions of the deciduomata where they split into finer units (Fig. 36).

The observations already recorded furnish considerable evidence

concerning the origin of the fibrillar material of deciduomata. The well developed deciduoma involves practically all of the endometrium, both mesometrial and antimesometrial, at the site of its formation. In rats 1 year of age, this endometrium is almost completely collagenous. At the periphery of the growth in the transitional zone there was evidence that the broad collagenous fibers were being divided into finer fibers and even fibrils, while in the interior of the decidual mass the fibers present appeared to be splitting into their component fibrils. We have therefore come to the conclusion that, during the development and growth of the deciduoma, the normal pattern of the endometrial connective tissue fibers, practically all of which are collagenous, is completely altered, apparently by a process in which the fibers are split into much finer elements which are generally transitional or reticular in nature. The fibrillar material is then reorganized into the pattern found in the deciduoma. The coarse fibers present are apparently those which have not been reduced to smaller units.

Deciduomata from Rats 3 Days after Uterine Irritation (Eighth Day of Pseudopregnancy)

The 31 deciduomata studied in this group were variable in size; some could scarcely be seen while others were as large as those in the 5-day group. Histologically, all of these, particularly the smaller, were less sharply delineated from the surrounding endometrium, the transitional zones being relatively wide in comparison with the size of the decidual masses themselves. Furthermore, structural changes in the endometrium adjoining the zone of transition were found, probably because this endometrium was destined later to be incorporated into the decidual mass.

As the deciduoma was approached from the normal uterus on either side, the first change usually noted was some increase in the width of the subepithelial zone of reticulum, usually most pronounced at the mesometrial pole. The most peripheral decidual reaction first appeared immediately under the lining epithelium in this region (Fig. 41). Progressing from this point inward, the decidual reaction appeared to radiate laterally and antimesometrially as well as mesometrially, and rapidly became larger (Figs. 15 and 18). As this occurred, more and more of the endometrium became involved. The collagenous connective tissue fibers became split into finer elements and became increasingly argyrophilic, although fairly wide fibers often extended quite a distance into the growth (Fig. 36). A mass of decidua was pushed into the lumen from the mesometrial side (Fig. 15); this presumably was the mechanism by means of which the uterine lumen was occluded. In

most of the smaller deciduomata the small-cell area, located mesometrially, involved more of the endometrium at proximal and distal extremities of the deciduoma than did the large-cell area situated on the antimesometrial side.

With two exceptions, both large and small decidual cells were present in the deciduomata of this group and, although there were some variations, the small cell-areas seemed better developed. In two very weak reactions, only small cells were present, both on the mesometrial side. These two do not necessarily indicate that this area is the normal site of the primary focus of decidual formation. In fact, Krehbeil, [15] who has studied deciduomata induced without mechanical injury, stated that the primary focus is on the antimesometrial side. Regardless of its mesometrial or antimesometrial situation, we believe, with Krehbeil, that the first decidual cells appear immediately under the lumen in the subepithelial reticular zone (Fig. 41).

The decidual cells, both small and large, in deciduomata of this group, were generally not as large as the corresponding types in the 5-day group. The condition of the fibrillar material, however, was similar, although the regular meshwork about the cells, as shown in Figure 27, was much more frequently found than in the 5-day group, and most frequently in areas fairly close to the lumen or its remnant. It should be remembered that in the normal uterus a regular reticular meshwork is found immediately underlying the lining epithelium (Fig. 21) and below this there is usually a meshwork of fairly thin collagenous fibers. Evidently the relatively more regular arrangement of the fibrillar elements of this area of the normal endometrium results in a more regular reticular meshwork about decidual cells that form here.

Changes in the endometrium immediately adjacent to the transitional zone were often striking. One type of change is shown in Figure 20. The light areas in the endometrium of this section represent sites at which the broad collagenous fibers (Fig. 23) commonly found in this region have disappeared and have been replaced by a sheet of finely fibrillar collagen which stained light blue in the Mallory preparations (Fig. 38) and a light shade of old rose by the Gömöri method (Fig. 37). Although the individual fibrils encountered in these areas were as thin as the finest reticular fibrils found in the deciduoma, they were collagenous rather than reticular. The significance of this finding will be discussed later. The stromal cells were moderately hypertrophied. In Figure 38, it is noted that remnants of the broad collagenous fibers persisted and that they stained more deeply than the finer fibrillated material surrounding them. In such areas of endometrium adjoining the decidual mass, the broad collagenous fibers around the glands

showed a marked tendency to persist (Fig. 20) while those more remotely situated from the glands were replaced by the finely fibrillated material.

In the zone of transition seen in Figure 20, the moderately hypertrophied stromal cells blended so gradually with those of the decidua that it was impossible to ascertain exactly where endometrium ended and decidua began. Here most of the broad collagenous fibers ordinarily found in the endometrium were replaced by generally thinner and more irregularly arranged elements.

Deciduomata from Rats 8 to 11 Days after Uterine Irritation (13th to 16th Days of Pseudopregnancy)

The 28 deciduomata from these rats were considered as a single group because of common structural characteristics; *i.e.*, degenerative changes and formation of the "metrial gland" (Fig. 40).

Degenerative changes in the decidual cells were found as early as 8 days after uterine irritation and became progressively more marked in the older growths. The changes occurred first in the large decidual cells bordering the remains of the uterine lumen and involved the smaller cells secondarily. As these changes progressed, the degenerated tissue was sloughed into the remains of the uterine cavity, which became larger as more and more decidua disappeared (Figs. 17 and 40). At points at which the degenerative process was complete, an epithelial lining reappeared in the lumen (Fig. 40). In most of the deciduomata of this group, the large cells had already disappeared but varying amounts of the small-celled decidua were found in all (Fig. 40). Rats killed during this period showed a bloody vaginal discharge similar to that found when spontaneous fetal resorption was occurring.[18]

The degenerative process involved shrinkage of the cells and pyknosis of the nuclei in both the large and the small decidual cells (Fig. 17). In the small-celled areas, the intercellular spaces disappeared and the cells became more closely packed. The sinusoids immediately under the lumen on the mesometrial side were generally distended. Often their walls were broken down and there was hemorrhage into the structureless mass lying in this region (Figs. 17 and 40). The type of degenerative process described here appeared to be similar to that which occurs in the placenta during spontaneous fetal resorption in the rat [18] and it is believed that the underlying mechanism is the same in both instances.

The appearance of the fibrillar material was somewhat variable and difficult to analyze. However, the structure of the fibers and fibrils and their affinity for the stain depended, at least to a certain extent, on the degree of cellular degeneration which had occurred in that particular

area. In those areas in which the degenerative process was not yet marked, the fibrillar material was not greatly different from that found in the 5-day group. However, since the intercellular spaces had largely disappeared, there was a greater irregularity in the relation of the fine fibrils to the cells.

In areas in which the process of degeneration had progressed to the stage of cellular disintegration (Fig. 17; immediately surrounding the lumen) the fibrillar substance showed pronounced and fairly constant changes. The thicker fibers lost their regular, compact appearance. Many fine fibrils projected from their lateral borders, giving them a shaggy appearance (Figs. 12 and 19; fibrils indicated by arrows). It appeared that the mechanism by which many fibrils are bound together to form a fiber had failed and that the fibers were breaking down into their component fibrils. Although these fibrils were extremely thin, they were usually collagenous (Figs. 12 and 19); however, in many instances their tips were argyrophilic (Fig. 12).

In other areas great numbers of fine fibrils formed large, irregular, tangled masses lying among, but not definitely associated with, the degenerate decidual cells. These fine fibrils usually stained rose-red; less frequently they were weakly argyrophilic. Fibrils of this size in a healthy deciduoma would almost invariably be reticular in nature. Therefore, in the degenerating decidual tissue, there was a distinct loss in the capacity of the fibrillar material to be impregnated by silver salts, although fine fibrils retained this capacity to a certain extent. Our observations would indicate that the degenerate fibrillar material was lost along with the necrotic cellular mass.

Beginning at about the same time as the degenerative changes described above, an enlargement occurred in the mesometrium (Fig. 40). The predominant cells in this swollen area were large, contained many red granules which were irregular in size and distribution and had irregular processes. These cells were arranged about the blood vessels forming a series of lobules (Fig. 40). This enlargement, called the "metrial gland" by Selye and McKeown,[17] has been described in detail by them. Therefore, our description will be limited chiefly to the fibrillar material.

In the normal mesometrial region (Fig. 16; taken from a 3-day deciduoma) blood vessels were abundant. They were surrounded by narrow collagenous fibers, although immediately beneath the endothelium reticular fibrils were present. Lying between the blood vessels were fine, closely spaced collagenous fibrils arranged in a fibrillated sheetlike mass. Stromal cells were situated between the perivascular collagenous fibers and in the finely fibrillated collagen between the vessels. In the 5-day deciduomata, these stromal cells, as well as those

lying between the muscle fibers of the circular smooth muscle in this region, were already hypertrophied. Hypertrophy in the latter situation caused a separation and some scattering of the muscle fibers. In the present group, this condition was marked and established a more or less direct connection between the metrial gland and the decidual mass (Fig. 40). These preliminary changes apparently preceded the formation of the well developed metrial enlargement.

The fibrillar material of a portion of a single lobule of the fully developed metrial enlargement is shown in Figure 42. The perivascular connective tissue was collagenous. Arranged about the cells was an irregular meshwork of fibrils and thin fibers. Occasionally the fibrillar material about the cells was definitely reticular (Fig. 42) but in most instances these fibers stained purplish or blackish red and were considered to be transitional in nature. Separating the lobules were more densely arranged and thicker collagenous fibers. Often the lobules were irregularly formed and not well separated from each other. Our studies would indicate that the fibrillar material in the metrial enlargement represented a modification of the connective tissue fibers, practically all of which are collagenous, normally found in the mesometrial region.

The origin and significance of the granular cells of the mesometrial region have been considered by Selye and McKeown.[17] These writers reported that they have a widespread origin and may arise from several types of cells found in this region; *i.e.*, smooth muscle cells, endothelial cells and fibroblasts. Although our observations are more limited, we think that their origin is not so general. As already indicated, all stromal cells in this region, including those lying between the blood vessels and those lying between the smooth muscle fibers, showed a definite hypertrophy, and in the rats with degenerating deciduomata it was possible to find transitional forms between the decidual cells and the granular cells. We are, therefore, of the opinion that these granular cells are derived only from connective tissue cells or from their derivatives.

Discussion

Two questions of interest arise as a result of these studies. They are concerned with the nature of the changes which occur in the fibrous material of the endometrium and the factors which determine the staining reaction of these connective tissue fibers.

It is well recognized that decidual cells arise as the result of hypertrophy and proliferation of the endometrial stromal cells. This study indicates that in like manner the fibrillar elements are derived from the endometrial connective tissue fibers which are profoundly modified

during the course of development of the deciduoma. There seems to be a close parallelism between the cellular and fibrillar changes; as the stromal cells hypertrophy the fibrillar material is completely reorganized into the pattern found in the deciduoma. Apparently this is accomplished by a division of the thick, endometrial, collagenous fibers into thinner fibers and fibrils, which become either transitional or reticular in nature. The finer elements usually come to be arranged in a more or less complete meshwork about the decidual cells. This study therefore presents definite morphologic evidence that endometrial collagen is transformed into the reticular and transitional fibers found in deciduomata. This view is further supported by two negative findings. There was only a very small amount of preëxisting reticulum in the endometrium in which the deciduomata were stimulated and we could find no evidence that this accounted for the large amount of reticular material in the decidual growths. There was also no evidence of the deposition of new fibrillar material which could account for the large amount of decidual reticulum. It is already generally recognized that reticulum may be transformed into collagen and these studies furnish histologic evidence that under certain circumstances collagen may be changed into reticulum. It would seem, therefore, that the fibrous, intercellular material of the endometrium is quite labile and subject to change under the proper conditions. It will be of great interest to see if this is true in connective tissue generally.

It has already been indicated that the exact relationship of reticulum and collagen is a controversial matter. Therefore, certain of our findings, which might shed some light on the subject, should be pointed out. In deciduomata a fairly definite relationship between the thickness of a fiber and its reaction to the silver stain was found. In normal decidual tissue the fibrils and thinner fibers were argyrophilic, while the thicker elements generally took a stain characteristic of collagen or transitional material. The same holds true in the normal endometrium; the fine elements immediately under the lining epithelium stained black and were considered to be reticulum, while the thick fibers deep in the endometrium were rose red, which indicated their collagenous nature. To a certain degree, therefore, our findings would tend to support the views of Mallory and Parker.[10] However, certain other observations indicate that the thickness of a fiber is not the only factor which determines whether it will or will not be impregnated with silver. In some deciduomata it was found that coarser fibers, up to 3 μ in thickness, were sometimes argyrophilic and thinner fibers of approximately 1 μ in width were quite regularly stained black. Fibers of these thicknesses in the normal endometrium were almost invariably collagenous. Furth-

ermore, in the normal uterus, fine collagenous fibrils have repeatedly been observed in the mesometrial region and in the spaces between the two muscle layers. In the collagenous zone of the normal endometrium fine fibrils were often seen in instances in which broad bandlike fibers split up into fine fibrils (Fig. 6); both were collagenous in spite of the tremendous difference in width. Furthermore, in degenerating decidual tissue, the finest fibrils showed a marked tendency to be collagenous in nature (Fig. 12).

These observations indicate that the location of a fibril or a fiber and its relationship to the surrounding tissues may play a definite rôle in its staining reaction. Thus in healthy decidual tissue there appears to be some influence which increases the capacity of the fibrous elements to be reticular, while in degenerating decidual tissue such an influence is evidently missing. In the normal uterus fine fibrils immediately adjacent to the lumen are reticular while those which are just as fine, but are located in the mesometrial region, are collagenous. We feel, therefore, that it is legitimate to suggest that some local environmental factor may play a rôle in the staining reaction of the connective tissue fibers. Although the nature of this hypothetical environmental factor is not known, our observations would suggest that it is associated with cell activity. Thus in degenerating decidual tissue even the finest fibrils tend to lose their argyrophilia and the degree of this loss appears to be associated with the degree of regression of the decidual cells. Such fibrils in healthy decidual tissue were invariably reticular. In the normal endometrium, the cellular elements are most abundant immediately under the lining epithelium; the fibrils here are definitely reticular. Yet fibrils just as thin, located in a zone where cells are less abundant, are collagenous.

If such an environmental factor actually exists, it seems possible that it is some metabolic product of the cell which is released into the surrounding tissues and is conveyed to the fibers by the intercellular fluid which bathes both fibers and cells. To carry this concept a step further (and we emphasize that the concept is purely hypothetical), it seems possible that this substance might penetrate into the fiber to a variable degree and thus modify its permeability or its chemical nature.* On this basis, it would be entirely logical for thin fibrils to be more argyrophilic than thicker fibers. Yet, if this environmental influence is strong enough, even thick fibers might be argyrophilic or transitional, and if it is sufficiently weak, even the finest fibrils would exhibit a

*N. C. Foot (On the origin of reticulin fibrils. *Am. J. Path.*, 1927, **3**, 401–412) has suggested that collagen is a colloidal or semifluid substance which permeates fibers and induces them to lose their affinity for the silver salt.

collagenous reaction. Although this hypothesis is not supported by direct evidence, it does form a logical explanation for our observations.

In certain deciduomata there was a considerable tendency for some transitional or collagenous fibers to have black lateral borders (Figs. 5, 7, 11 and 13). As far as we can ascertain, such a finding has not been reported before. Since we have observed this phenomenon to a slight extent in previous studies on endometrial connective tissue and with considerable regularity in the present material, it should be mentioned. Although the significance of this phenomenon is unknown, it seems obvious that it is most easily interpreted on the basis of the hypothesis described above, namely, that the outer surfaces of the fibers are more accessible to the environmental influence and would therefore react more readily to the silver salts.

Summary and Conclusion

1. The fibrillar material in deciduomata of the rat has been studied by methods in general use for the differentiation of reticulum and collagen.

2. The fibrillar elements ranged from fine fibrils to fibers approximately 3 μ in width. The fine fibrils were almost always reticular in nature. The thick fibers were sometimes reticular, but more often they were either collagenous or transitional between reticulum and collagen. It was found that the fibrous material in deciduomata of the rat was derived from that of the normal endometrium which underwent marked changes during the development of the decidual growth. Evidence is presented to show that under certain circumstances collagen may be transformed into reticulum.

3. The relationship between reticulum and collagen is discussed and factors regulating their staining reactions are considered. It is suggested that certain localized factors might influence the staining reaction of connective tissue fibers.

References

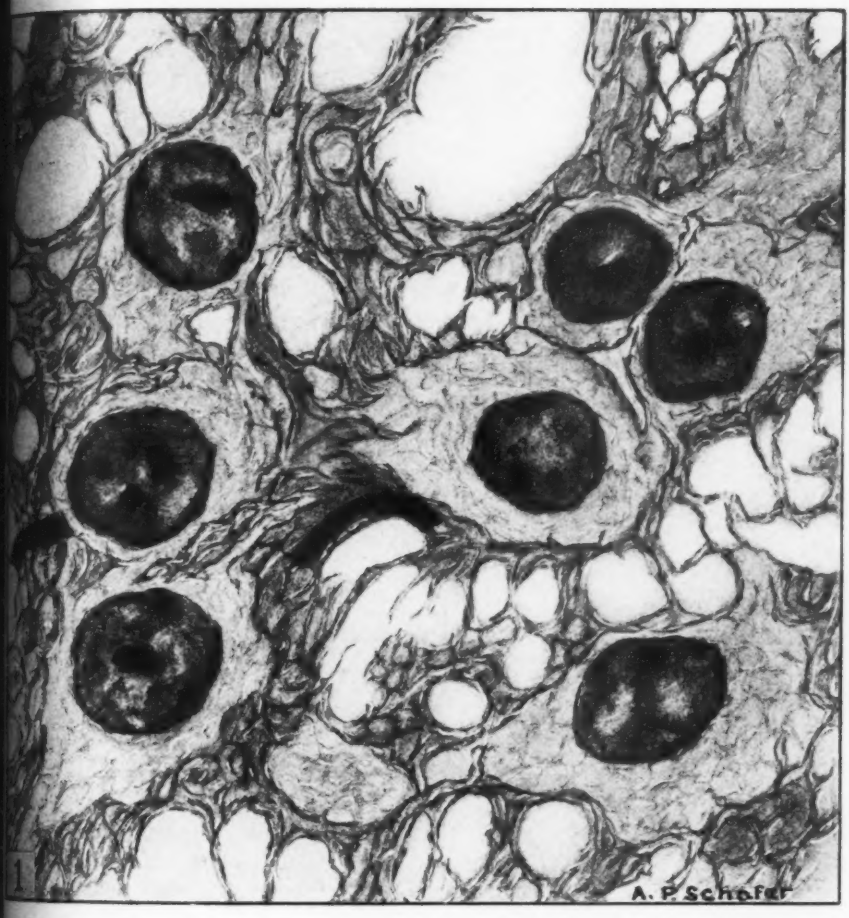
1. Wolfe, J. M.; Burack, Ethel; Lansing, Winifred, and Wright, A. W. Changes in the connective tissue of the uterus and vagina of the rat associated with advancing age. *Science*, 1940, **91**, 577–578.
2. Wolfe, J. M.; Burack, Ethel; Lansing, Winifred, and Wright, A. W. The effects of advancing age on the connective tissue of the uterus, cervix and vagina of the rat. *Am. J. Anat.*, 1942, **70**, 135–165.
3. Mall, F. P. Das reticulierte Gewebe und seine Beziehungen zu den Bindgewebsfibrillen. S. Hirzel, Leipzig, 1891. Repr. from: *Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissensch., Leipzig*, 1891, **17**, 299–338.
4. Mall, F. P. Reticulated tissue, and its relation to the connective tissue fibrils. *Johns Hopkins Hosp. Reports*, 1896, **1**, 171–208.

5. Kupffer, C. Ueber Sternzellen der Leber. *Arch. f. mikr. Anat.*, 1876, **12**, 353–358.
6. Oppel, Albert. Eine Methode zur Darstellung feinerer Strukturverhältnisse der Leber. *Anat. Anz.*, 1890, **5**, 143–145.
7. Maresch, Rudolf. Ueber Gitterfasern der Leber und die Verwendbarkeit der Methode Bielschofskys zur Darstellung feinster Bindegewebsfibrillen. *Centralbl. f. allg. Path. u. path. Anat.*, 1905, **16**, 641–649.
8. Rössle, Robert, and Tanzo, Yoshida. Das Gitterfasergerüst der Lymphdrüsen unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, **45**, 110–126.
9. Russakoff, A. Über die Gitterfasern der Lunge unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1909, **45**, 476–506.
10. Mallory, F. B., and Parker, Frederic, Jr. Reticulum. *Am. J. Path.*, 1927, **3**, 515–525.
11. Foot, N. C. Chemical contrasts between collagenous and reticular connective tissue. *Am. J. Path.*, 1928, **4**, 525–544.
12. Miller, W. S. The reticulum of the lung. 1. Its relation to early tuberculous processes. *Am. Rev. Tuberc.*, 1923, **7**, 141–160.
13. Foot, N. C. On the origin of reticulin fibrils. *Am. J. Path.*, 1927, **3**, 401–412.
14. Maximow, Alexander. Development of argyrophile and collagenous fibers in tissue cultures. *Proc. Soc. Exper. Biol. & Med.*, 1927–28, **25**, 439–442.
15. Krehbeil, R. H. Cytological studies of the decidual reaction in the rat during early pregnancy and in the production of deciduomata. *Physiol. Zool.*, 1937, **10**, 212–233.
16. Gömöri, G. Silver impregnation of reticulum in paraffin sections. *Am. J. Path.*, 1937, **13**, 993–1001.
17. Selye, H., and McKeown, T. Studies on the physiology of the maternal placenta in the rat. *Proc. Roy. Soc., London, s. B.*, 1935–36, **119**, 1–31.
18. Burack, Ethel; Wolfe, J. M., and Wright, A. W. Prolonged vaginal bleeding and fetal resorption in the Albany strain of rats. *Anat. Rec.*, 1939, **75**, 1–17.

DESCRIPTION OF PLATES

PLATE 69

FIG. 1. Pencil drawing of a section stained by the Mallory technic showing transitional decidual cells surrounded by intercellular material which in such preparations did not appear definitely fibrillar. Large intercellular spaces and portions of two thick fibers are seen. × 1950.



Wolfe and Wright Connective Tissue of the Induced Placenta

PLATE 70

(All figures taken from Gömöri preparations of deciduomata unless otherwise noted.)

FIG. 2. Fine reticulum fibril splitting into finer units. × 1200.

FIG. 3. Fine reticulum fibril splitting into finer units. × 1200.

FIG. 4. Portion of a thick transitional fiber showing splitting into two thinner elements. × 1200.

FIG. 5. A thinner transitional fiber. The lateral surfaces are argyrophilic. × 1200.

FIG. 6. A collagenous fiber from normal endometrium, showing one end split into thin fibrils which are collagenous in nature. × 1200.

FIG. 7. A thick (3 μ) transitional fiber which shows argyrophilic fibrils splitting off at one end and a dark surface area. × 1200.

FIG. 8. A transitional fiber almost as black as reticulum. The thinner elements separating from the fibers are definitely argyrophilic. × 1200.

FIG. 9. A thick (3 μ) reticular fiber beginning to split into finer elements. × 1200.

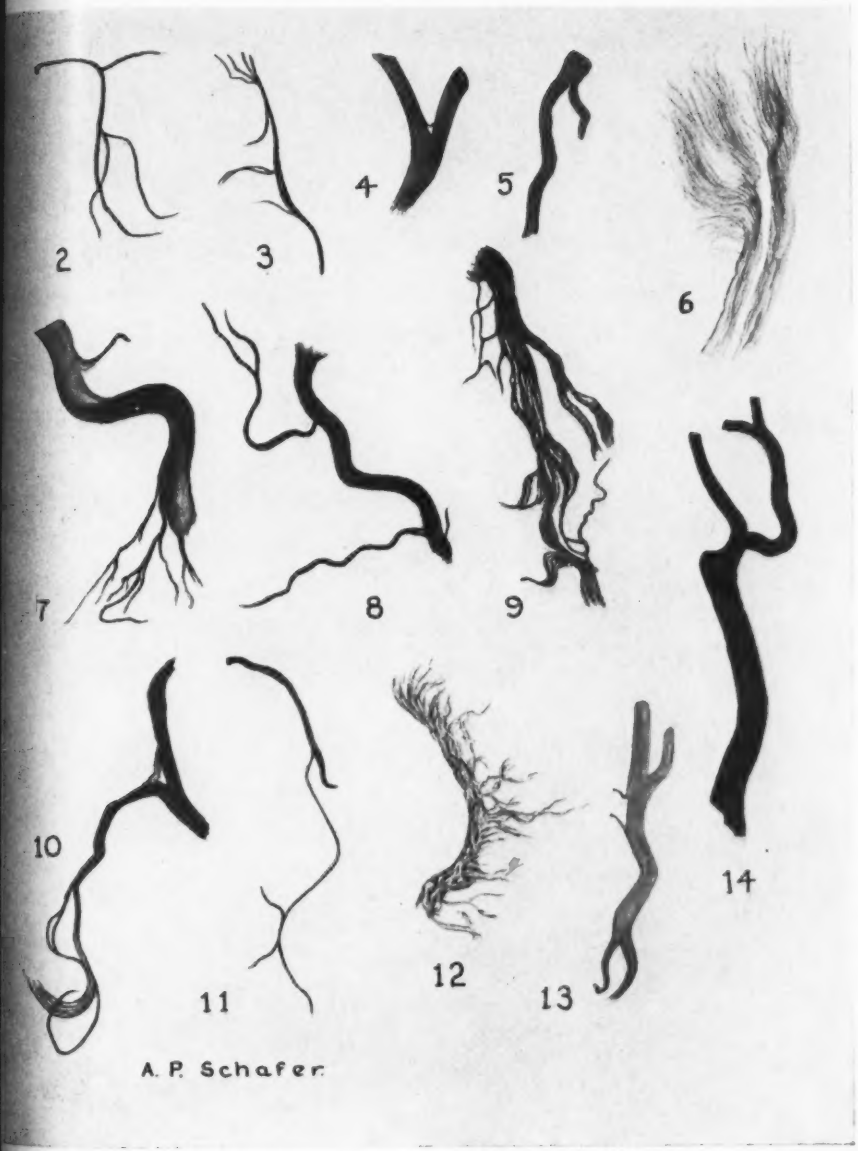
FIG. 10. A moderately thick reticular fiber with finer branches. × 1200.

FIG. 11. A thin transitional fiber showing a fine reticular fibril coming off one side. × 1200.

FIG. 12. A collagenous fiber taken from degenerating decidual tissue (see Fig. 17). The constituent fibrils are separating. Although the fibrils are stained rose-red, their tips are weakly argyrophilic. × 1200.

FIG. 13. A collagenous fiber (2 μ) found in normal decidual tissue. × 1200.

FIG. 14. A thick (slightly over 3 μ) reticular fiber. × 1200.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A. P. Schafer

PLATE 71

FIG. 15. A small 3-day deciduoma. Mallory preparation. × 18.

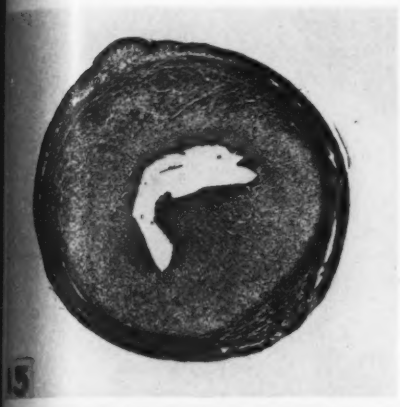
FIG. 16. Mesometrial region of 3-day deciduoma. Mallory preparation. × 62.

FIG. 17. Degenerating deciduoma showing large and small cells. There is a hemorrhage and a necrotic mass immediately under the lumen. Mallory preparation. × 115.

FIG. 18. Small 3-day deciduoma. Blending of the fibrillar material of the decidual growth can be seen, which is located mesometrially with that of the adjoining endometrium. Gömöri preparation. × 62.

FIG. 19. Section from degenerating deciduoma. Irregular collagenous fibers can be seen (see arrows) lying among the cellular débris. Gömöri preparation. × 720.

FIG. 20. Section from a 5-day deciduoma showing the junction of the decidual mass with the endometrium. The changes shown here may be compared with Figure 24. Mallory preparation. × 62.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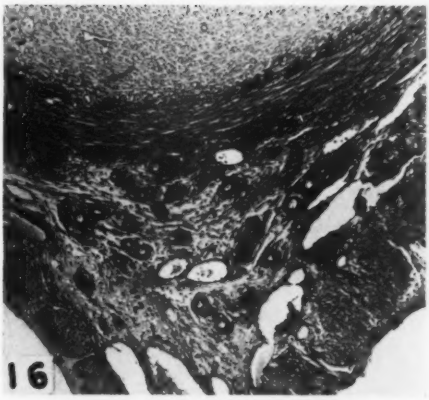
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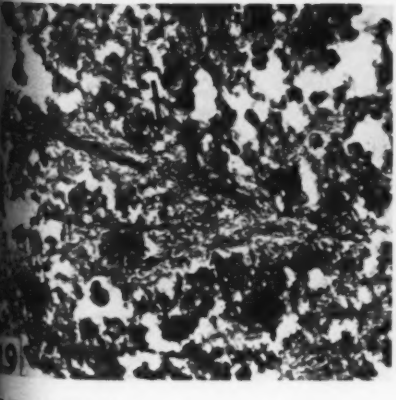
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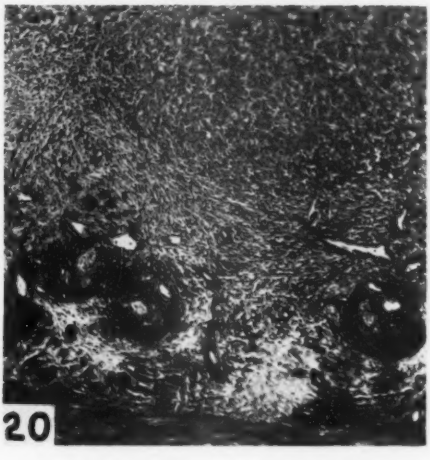
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PLATE 72

FIG. 21. Normal endometrium showing the lining epithelium, the basement membrane and the subepithelial reticular zone. The reticulum gradually blends into the underlying collagen, which appears gray. Gömöri preparation. × 168.

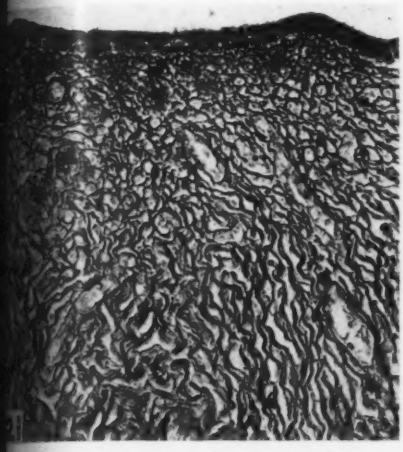
FIG. 22. Normal endometrium showing the lining epithelium and the abundant stromal cells in the subepithelial reticular zone. There is thickening of fibers deeper in the endometrium. Mallory preparation. × 168.

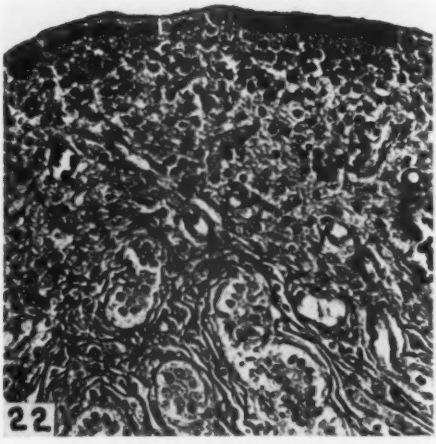
FIG. 23. Outer portion of normal endometrium lying next to circular smooth muscle. Note thickness of collagenous fibers lying parallel to the muscle and around the glands. Compare with Figure 21. Gömöri preparation. × 168.

FIG. 24. Outer portion of normal endometrium adjoining the circular smooth muscle. Note the broad fibers and relative inabundance of stromal cells. Compare with Figure 22. Mallory preparation. × 168.

FIG. 25. Small decidual cells. Mallory preparation. × 280.

FIG. 26. Large decidual cells. Mallory preparation. × 280.

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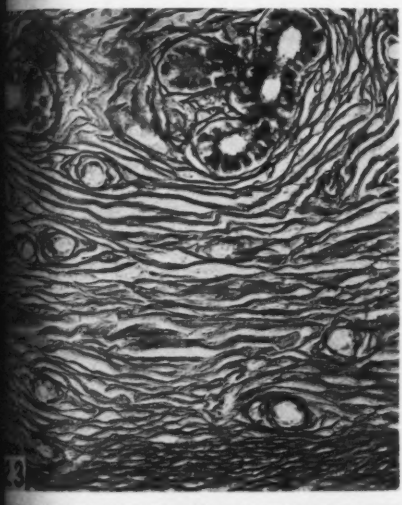

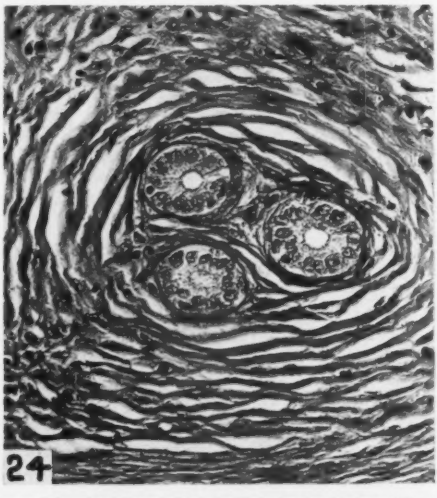
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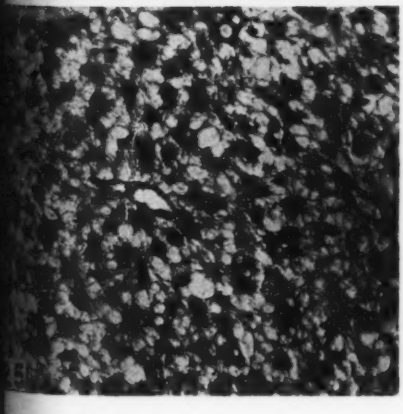

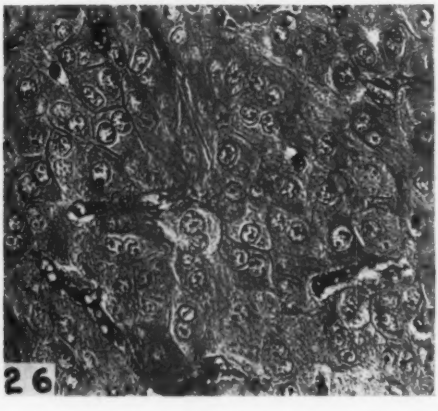
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PLATE 73

(All figures taken from Gömöri preparations.)

FIG. 27. Section from small-decidual-cell area showing a very regular reticular meshwork. × 600.

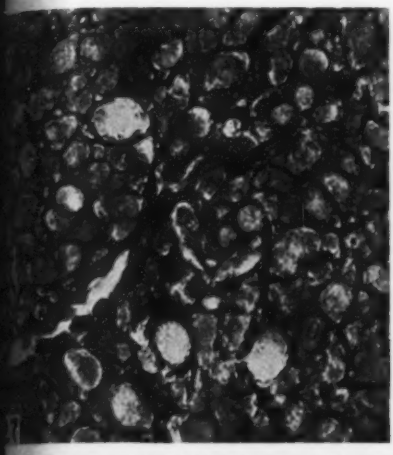
FIG. 28. Section from small-decidual-cell area showing intercellular spaces and fine reticular fibrils closely applied to the cells. There are also thick fibers which are reticular. × 600.

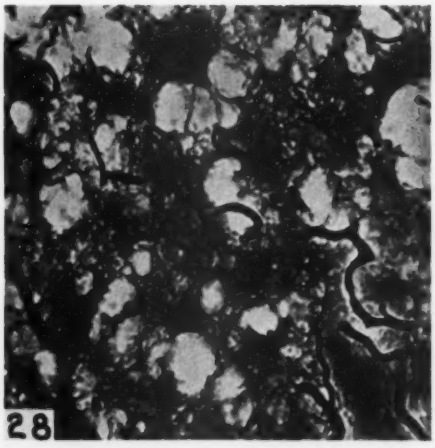
FIG. 29. Section from small-decidual-cell area showing smaller intercellular spaces and less abundant reticulum. Here there are the thicker fibers. × 600.

FIG. 30. Section from near periphery of small-decidual-cell areas showing an irregular arrangement of cells, intercellular spaces and fibrillar material. × 600.

FIG. 31. Section from near periphery of small-decidual-cell areas. There are many branching fibers and fibrils, nearly all of which were reticular. × 280.

FIG. 32. Section from near periphery of small-decidual-cell areas showing an irregular arrangement of fibrillar material; the thicker fibers were transitional. × 280.

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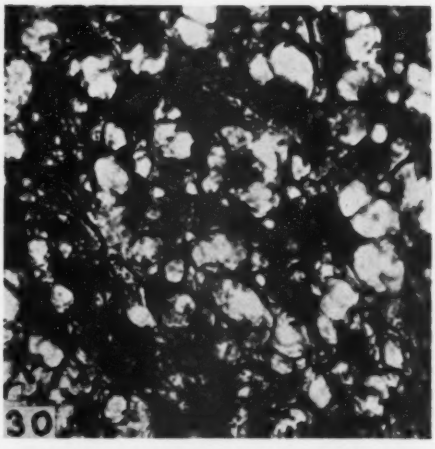
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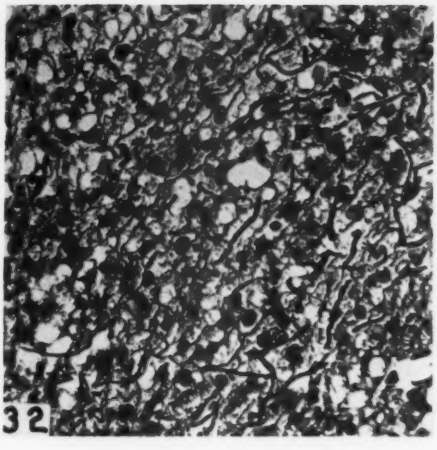
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PLATE 74

(All figures are from Gömöri preparations unless otherwise noted.)

FIG. 33. Section from large-decidual-cell area showing scant and irregularly arranged fibrillar material which was reticular. × 600.

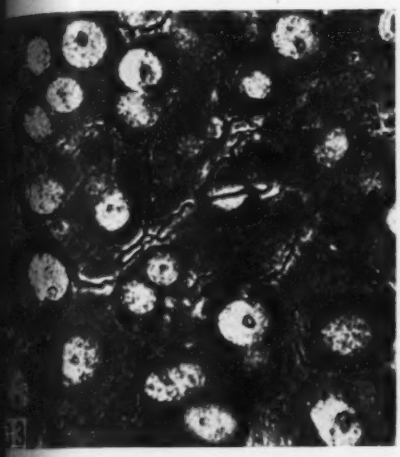
FIG. 34. Large decidual cells showing abundant fibrillar material which was reticular. × 280.

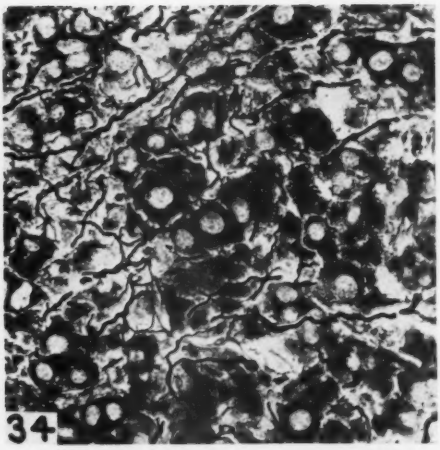
FIG. 35. Junction of a 5-day deciduoma with normal endometrium. × 96.

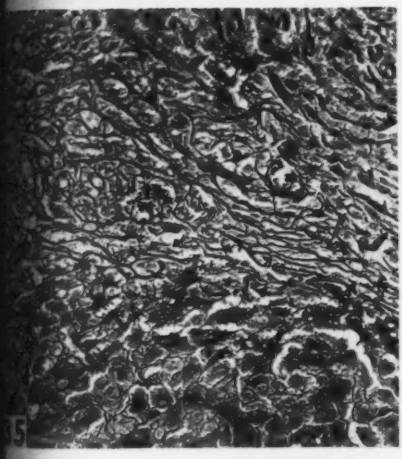
FIG. 36. Junction of a 3-day deciduoma with normal endometrium showing very gradual transition and broad collagenous fibers extending into the deciduoma. × 96.

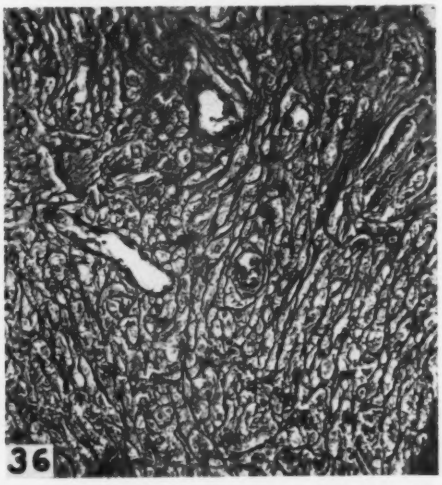
FIG. 37. Section from the outer portion of the endometrium adjoining a deciduoma. Here the thick collagenous fibers, such as those seen in Figure 23, have been replaced by a finely fibrillated collagen. Stromal cells lie imbedded in the collagenous tissue. The circular smooth muscle is shown. × 168.

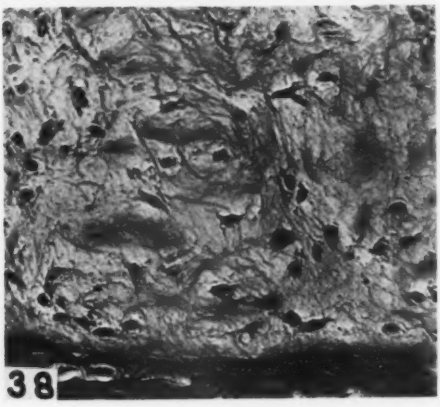
FIG. 38. A Mallory preparation taken from an area comparable to that in Figure 37. The finely fibrillated nature of the collagen is evident. The darker and more compact areas represent persisting, thicker, collagenous fibers. The circular smooth muscle also is shown. × 168.











...dle and Wright Connective Tissue of the Induced Placenta

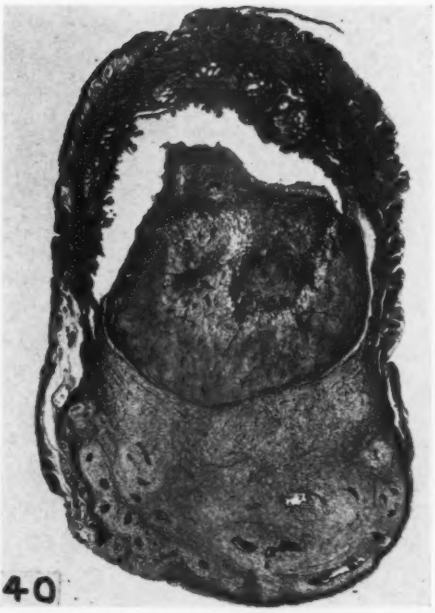
PLATE 75

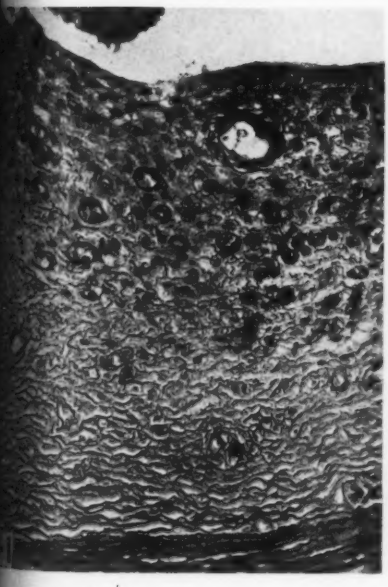
FIG. 39. Section through a 5-day deciduoma. The small- and large-decidual-cell areas may be recognized at the mesometrial and antimesometrial poles, respectively. There is a blood-filled space near the center. Mallory preparation. × 16.

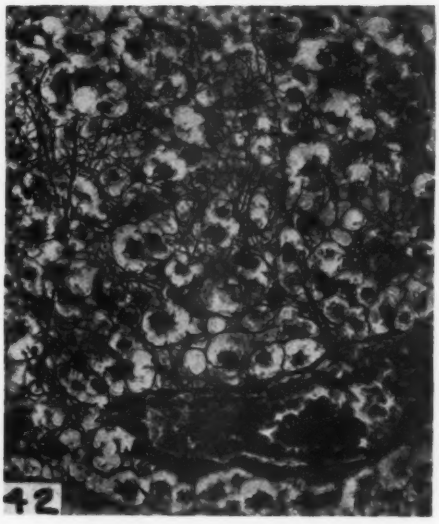
FIG. 40. Section through a 10-day deciduoma showing degenerative changes. The large-cell portion of the decidual mass has disappeared and part of the small-cell area has been replaced by a structureless mass. The uterine cavity has reappeared and is lined by epithelium on the antimesometrial side. The mesometrial region may be compared with that in Figure 39. Mallory preparation. × 16.

FIG. 41. Section through the peripheral portion of a weak 3-day decidual reaction. There is moderate hypertrophy of the stromal cells in the subepithelial reticular zone and the process is progressing into the deeper endometrium. There is reticulum in areas where stromal cell hypertrophy has occurred. This reaction occurred at the mesometrial pole. Gömöri preparation. × 181.

FIG. 42. A portion of one lobule found in the mesometrial enlargement (metrial gland). The central blood vessel is surrounded by collagen while reticular fibers surround the large granular cells. Gömöri preparation. × 181.







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Connective Tissue of the Induced Placenta

EXPERIMENTAL BLASTOMYCOSIS IN MICE *

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In previous papers I have shown that the virulence, for mice, of the causative organisms from two cases of human blastomycosis was not enhanced by mouse passage,[1] and that the filamentous form was as infectious for mice as the yeast form.[2]

Since blastomycosis was being produced very consistently in this convenient laboratory animal, observations were made on the reactions of the tissues of the mouse to this large fungous organism. This study was augmented with an attempt to explain some of the tissue responses by noting the results of injections into mice of killed blastomycetes and of a phosphatide fraction of the fungus. Rabbits were utilized for an accompanying study of the effect of a polysaccharide fraction on the tissues. The chemical fractions were obtained from Peck and Hauser, whose published papers about the fractions may be consulted.[3,4]

LITERATURE

In all of these reports the injected organisms were obtained from proved human cases of blastomycosis as recently tabulated by Martin and Smith.[5]

Bowen and Wolbach [6] inoculated four mice intraperitoneally and obtained satisfactory abdominal and pulmonary lesions. They stated: "The type of lesion in the lung, the filling of the alveoli with large cells and organisms with little other reaction, is a peculiar one and deserves further study." Photographs show sections of the lesions of the lung of a mouse killed 40 days after intraperitoneal inoculation. The abdominal lesions had largely disappeared. DeMonbreun [7] found, from injection of yeastlike forms of the organisms, that "each of six mice died in from three to five weeks after inoculation, and numerous small abscesses containing the fungous cells were found in the lungs, liver, spleen and kidneys." In mice inoculated by Bergstrom, Nugent and Snider,[8] profuse lesions developed within 5 weeks, but the animals did not die until 10 weeks after inoculation. Davis [9] found that blastomycetes injected into the peritoneal cavity of guinea pigs were rapidly taken up by leukocytes, macrophages principally.

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Materials and Methods

Two strains of *Blastomyces dermatitidis* were employed, the sources of which have been previously detailed.[1] Strain A was from a fatal case of systemic blastomycosis and strain B was from a cutaneous lesion which responded well to iodine therapy. Both strains were found to be capable of producing generalized blastomycosis in mice, and they are not differentiated in Table I.

The mice were inoculated intraperitoneally under rigid, aseptic technic with doses of pure cultures which varied from 0.5 cc. of a 1:200 suspension to 1 cc. of a 1:25 suspension, by volume. The yeast form of the organism was centrifuged in a calibrated tube at a standard speed and duration of time. Proper dilutions were made by adding saline solution. To obtain data to compare with the amounts of dead organisms and chemical fractions, the wet centrifuged blastomycetes were weighed, after the supernatant fluid was decanted. Another weighing after vacuum drying indicated that 1 cc. of the moist organism was equivalent to 52 mg. in the dry state. On this basis the dry weight injected varied from 0.1 to 2 mg. in different animals. The smaller dose was capable of producing generalized lesions, and no separation of the animals according to dosage has been made. Comment and data on the dosage factor are available in another report.[1]

Studies of the peritoneal fluid were made in mice sacrificed during the first 11 days following injection. The normal mouse has insufficient peritoneal fluid for cellular studies. Consequently, 0.5 cc. of physiologic saline solution was injected intraperitoneally just before the animal was killed. Total white counts were determined. The disposition of the blastomycetes and the comparative numbers of the cell types were determined by supravital study with neutral red and janus green, in a hot box, and by stained smears. Paraffin sections were found helpful in differentiating cell types, and gave topographic relations.

Thomas and Dessau [10] studied the cells of the peritoneal fluid in normal mice after rinsing the peritoneal cavity with 0.5 cc. of saline solution as just mentioned. The total cells averaged 5,900 per cmm. Undifferentiated cells made up 65.5 per cent in the differential counts. These "were small cells, about the size of the blood lymphocytes, with a small amount of cytoplasm and no constant cytoplasmic inclusions which stained with vital dyes." Monocytes numbered 32 per cent; polynuclear leukocytes, 1.5 per cent; basophilic leukocytes, 0.8 per cent, and clasmatocytes, 0.2 per cent. This classification has been followed in these experiments.

TABLE I

The Cellular and Tissue Response to the Injection of Living Blastomyces dermatitidis into the Peritoneal Cavities of Mice*

Duration	No. mice	No. died	No. sacrificed	Peritoneal fluid						Gross
				White cells	Neutrophils	Undifferentiated cells	Monocytes	Eosinophils	Organisms	
				per cmm.	%	%	%	%		
½ to 3 hrs.	2	0	2	6,000	19	70	11		Free	Normal
4 hrs.	2	0	2	9,300	83	14	3		Surrounded by neutrophils	Normal
1 day	2	0	2	19,600	56	17	24	3	Surrounded by neutrophils	Normal
3, 4, 5 days	3	1	2	9,500	27	52	21		Surrounded by neutrophils and monocytes; fibroblasts present	Dull peritoneum
1 to 5 wks.	59	50	9	5,000†	54	16	29	1	Surrounded by neutrophils and monocytes; blastomycetes rare	Nodules on peritoneum and usually in lungs
5 to 8 wks.	4	2	2							Nodules on peritoneum and usually in lungs
Total	72	53	19							

* Dose varied from: 0.5 cc. of 1:200 to 1 cc. of 1:25 suspension by volume, or 0.1 to 2 mg. dry weight.
† Average of 3 mice: 8, 10, 11 days.

Experimental Data

Living blastomycetes were injected into the peritoneal cavities of 72 mice. The great majority of mice died between 1 and 5 weeks after inoculation (Table I) and presented peritoneal and pulmonary nodules, and usually microscopic lesions elsewhere. Most of the mice which died became less active and lost weight during the several days before death.

During the first 3 hours the injected blastomycetes lay free in the abdominal cavity and the white and differential counts were those of normal mice. After several hours (Fig. 1) the blastomycetes were found surrounded by polymorphonuclear neutrophils. The peritoneal fluid also showed an increase in the number of white cells and polymorphonuclear neutrophils predominated instead of undifferentiated cells.

At the end of 1 day the organisms were surrounded by cells which were difficult to identify. In the supravital preparations the cytoplasm contained numerous, neutral-red vacuoles, and nuclear staining sometimes occurred, which, in such preparations, indicates dead cells. The nucleus was swollen and usually was not polymorphous. In the stained films the cytoplasm was like that of neutrophils. Study of paraffin sections suggested that most of these surrounding cells were probably injured polymorphonuclear neutrophils with swollen nuclei. The same difficulty was experienced in interpreting the nature of the surrounding cells throughout the 11-day period during which observations of peritoneal fluid were made. No such difficulty was experienced in making differential counts of the peritoneal fluid. The total white count was definitely elevated and the polymorphonuclear cells predominated in the differential count. The coating on the peritoneum was found in paraffin sections to consist of blastomycetes, interspersed with polymorphonuclear neutrophils.

In the period from 3 to 5 days the blastomycetes were surrounded by cells which were judged to be polymorphonuclear neutrophils, but some stimulated monocytes and occasional fibroblasts were noted. The peritoneal surfaces were dull to the naked eye, and occasional unattached white nodules 1 mm. in diameter were seen. At 5 days (Fig. 3) the peritoneal coating of blastomycetes and interspersed polymorphonuclears had become necrotic centrally in the nodules, but peripherally the blastomycetes were proliferating and attracting polymorphonuclear neutrophils. Karyorrhexis among dead polymorphonuclear neutrophils was often noted. Monocytes were present but not prominent. Fibroblastic outgrowth from the serosa was well developed and vascularization had begun.

In the period from 1 to 5 weeks, free blastomycetes in the peritoneal fluid were less numerous. They occurred in a mass of cells which were

thought to be predominantly injured polymorphonuclear neutrophils, though some were monocytes. Fibroblasts extending from such a mass at the end of 8 days are shown in Figure 2.

At 1 week white nodules and plaques were visible over the peritoneal and omental surfaces, and these continued to increase in size.

The gross appearance typical of the period between 1 and 5 weeks has been shown in previous papers.[1,2] Often a single, large, caseous nodule occurred between stomach and spleen, and smaller nodules elsewhere. At 1 week blastomycetes had been first noted in the retrosternal lymph nodes and in the lungs. From then on involvement of the lung was usually prominent and was noted in 90 per cent of the animals which died in this period (Fig. 5; also Fig. 4 in previous paper [2]). The route from the peritoneal cavity to the lungs was never by direct extension through the diaphragm because the lungs were free in the pleural cavities. The uniform distribution of nodules in the lungs indicated an hematogenous spread, either via the retrosternal lymphatics and nodes to the venous system, or directly from the peritoneal cavity into the vascular system. Hematogenous spread to cardiac muscle (Fig. 5 in previous paper [2]), brain, spleen and liver was demonstrated microscopically in a number of mice, and probably would have been demonstrated much more frequently if all of these organs had been systematically studied microscopically. An interesting involvement of the liver consisted of growth of organisms into portal veins with the production of large areas of infarction.

Microscopically, this period was characterized by the great proliferation of blastomycetes, whether in the abdominal cavity, lungs (Fig. 6) or elsewhere. There was little reaction of any kind in some masses but usually the blastomycetes were interspersed with polymorphonuclear cells (Fig. 6), fragments of nuclei, or less frequently with monocytes, and very rarely with the undifferentiated cells. Necrosis of the centers of blastomycetic masses was the rule; and fibroblastic growth developed at the periphery after a time, but generally the cellular reaction was less conspicuous than the blastomycetic proliferation.

Microscopic abscesses like those of human cutaneous blastomycosis rarely occurred. This may have been due to the fact that the blastomycetic growth was so massive and diffuse that microscopic collections of a few blastomycetes with surrounding polymorphonuclear cells were not frequent. Figure 4 shows a microscopic "abscess" adjacent to the pancreas in a mouse injected 11 days before.

Phagocytosis of blastomycetes by macrophages and giant cells was never a frequent appearance but it did occur, especially in the animals which had been injected for the longer intervals. Several giant cells

containing blastomycetes were noted in a mouse injected 34 days before.

In the few mice examined in the interval between 5 and 8 weeks, the central areas of blastomycotic masses were not only necrotic, but the outlines of the blastomycetic shells had disappeared, so that the appearance of caseation was produced. Dense fibrosis occurred about such areas.

"Toxicity" of Process

Some of the sacrificed animals were active and appeared healthy, but showed extensive abdominal and pulmonary lesions. It is not clear, therefore, that blastomycetic infection in itself is especially productive of a toxic state. The same may be said for human cases of blastomycosis, except in the terminal stages of the process. As possible causes for death in the mouse, aside from a toxic effect of the organism or of the products of disintegration of the lesions, are the presence of blastomycosis of the peritoneum which may interfere with peristalsis of the intestines, or blastomycosis of the liver which may interfere with the function of that organ. The arguments in favor of the toxicity of dead organisms will become apparent in the following section on killed blastomycetes.

Summary of Changes

The intraperitoneal inoculation of mice with *B. dermatitidis* led to a massive growth of organisms in the peritoneum with dissemination to the lungs and elsewhere. The blastomycetes attracted cells, chiefly polymorphonuclear neutrophils, and when the organisms occurred in masses they tended to develop necrotic centers. The early, peripheral, fibroblastic growth developed into an encapsulating, fibrous layer. The monocyte and giant cell appeared to be less important in the mouse than the polymorphonuclear neutrophil and the fibroblast.

Injection of Killed Blastomycetes

Killed blastomycetes were injected into the peritoneal cavities of 14 mice to determine, first, how closely the tissue response to the living organisms could be duplicated, and second, how toxic the suspension was.

A single small dose of killed organisms produced no apparent effect. Mice which had received 0.1 mg. were sacrificed after 8 weeks. No gross or microscopic abnormality could be demonstrated. It has already been shown that this amount of living fungus produced progressive blastomycosis and death.

To duplicate the effects of the proliferating, living yeast cells, repeated large doses of killed organisms were used. Ten mg. were given

every other day. (Dosage is in terms of equivalent dried weight of suspension.) Killing was effected by heating at 60° C. for 2 hours. After each aspiration of suspension from the rubber-stoppered vaccine bottle, the heating was repeated to insure sterility.

The mice tolerated the large doses poorly, and appeared ill after injections. Most of them died in 5 to 11 days, apparently of the toxic effects of the suspension.

In paraffin sections the fact that the fungus was dead was clearly evident in the failure of the blastomycetes, especially the internal substance, to stain with hematoxylin. Uniform staining with eosin occurred.

The peritoneum was lined with a friable, yellow layer. This was composed largely of the deposit of killed blastomycetes (Fig. 7). Between them, as between the living organisms in the first experiments, polymorphonuclear cells and a few mononuclear cells occurred. There was the same necrosis in the centers of large blastomycotic masses as was noted with the living organisms; but with the killed blastomycetes the necrosis applied only to the infiltrating cells, since the blastomycetes themselves were already dead. Eosinophils, and macrophages and giant cells containing blastomycetes, were more abundant than in the mice which received the living organisms. In the mouse examined 11 days after injection the outlines of the blastomycetes had largely disappeared, leaving "caseous" areas.

Pulmonary lesions were not produced. The retrosternal lymph nodes contained very large, finely granular reticulo-endothelial cells, but no blastomycetes.

In comparison with the living organism it was concluded that the initial cellular response, the central necrotizing process and the peripheral, fibroblastic response were similar. The early toxicity of the large doses of killed organisms was correlated with the toxicity of the living organisms after several weeks. By this time the formerly viable masses had become necrotic centrally and had probably liberated some toxic substance in the nature of an autolysate. Enzymes from the dead polymorphonuclear neutrophils may have produced the necrosis.

Blastomycetic Phosphatide in Mice

For the method of extraction and an account of the properties of this phosphatide the paper by Peck and Hauser [3] may be consulted.

Thomas and Dessau,[10] who studied the effects of chemical fractions of the tubercle bacillus in mice, stated: "It is of interest that while in guinea pigs and in rabbits the reaction to tuberculo-phosphatide resembles very closely the lesions produced by the living organisms, this mimicry is best accomplished in the mouse by the injection of the waxy

fractions." They found that the phosphatides of the tubercle bacillus stimulated the multiplication and partial maturation of monocytes. The formation of true epithelioid cells was not the rule.

The blastomycetic phosphatide had been passed through a Berkefeld filter for sterilization when it was in the stage of ether extraction. For injection of mice, 10 mg. were suspended in 1 cc. of distilled water. Microscopically the suspension showed droplets of which none was as large as a human red blood cell. After the suspension stood for a day, a small amount of material settled out.

Seven mice received the phosphatide intraperitoneally, in amounts varying from a single dose of 10 mg. to daily doses of 30 mg. for 24 days, with sacrifice 3 days later. The higher dosage resulted in prominent, white, peritoneal plaques.

A peritoneal reaction, predominantly of the monocytic series of cells, was seen in sections. Most of these cells were of moderate size. A few had fine vacuoles, some of which, in frozen sections, absorbed a little scharlach R. In addition to the predominant "large mononuclear cell," polymorphonuclear neutrophils and eosinophils, fibroblasts and reticulum were occasionally noted, the latter in mice allowed to survive for the longer periods.

Apparently, then, the phosphatide of the blastomycete acts on the mouse in much the same manner as does the phosphatide of the tubercle bacillus.

The significant point here is that the large majority of reacting cells were of the monocytic series (Fig. 8) rather than of the polymorphonuclear series. Moreover, a layer of the same thickness caused by the living or killed blastomycetes would show necrosis centrally, as was demonstrated by direct comparisons. Hence the phosphatide is not the chemical fraction responsible for the polymorphonuclear reaction and the necrosis, the two most striking features in connection with the living or killed organisms.

Further studies with chemical fractions have not been pursued in the mouse.

Blastomycetic Polysaccharide in Rabbits

A polysaccharide from *B. dermatitidis* was injected intraperitoneally into rabbits, because repeated samples of blood are easier to obtain in rabbits than in mice, and because a report on the effects of polysaccharides of certain bacteria on rabbits was already available for comparison.[11] Preparation 1 as described by Peck, Martin and Hauser[4] was used.

During the first few hours after the injection of 10 mg. in distilled

water there were produced sterile peritonitis, retrosternal lymphadenitis and the impressive changes in cells of the peripheral blood stream shown in Chart 1. These changes in the white blood cells consisted of (1) almost immediate leukopenia, (2) lymphopenia reaching the lowest point in 5 or 6 hours and (3) a great increase in immature polymorphonuclears (amphophils) which reached the maximum in 5 or 6 hours. The curve of these immature, non-filamented polymorphonuclears in Chart 1 ascends and crosses the curve of the more mature, filamented polymorphonuclears. This is thought to indicate that the bone marrow was stimulated to send these immature forms into the blood stream.

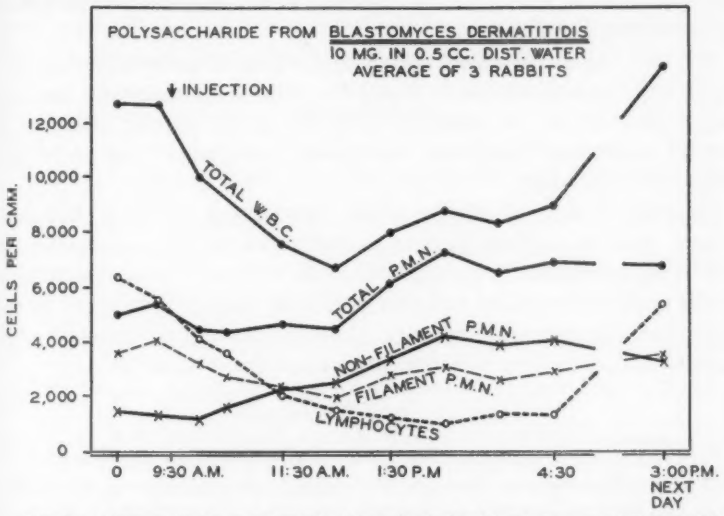


Chart 1. Changes in the number of white cells in the blood streams of three rabbits which received a polysaccharide of *Blastomyces dermatitidis* intraperitoneally.

The transient nature of the changes in the levels of the blood cells is indicated in Chart 1. They tended to return to the starting points on the second day when the rabbits were killed. Each of the individual graphs from which this average graph was constructed showed the same trend in lymphocytes and in non-filamented (immature) amphophils. The curve of the non-filamented forms of each rabbit crossed and went above the curve of the filamented forms.

Control studies in 13 rabbits injected intraperitoneally with distilled water, dextrose, saline solution, barium sulfate and lamp black, indicated that the described reactions in the blood stream were not specific for the blastomycetic polysaccharide, but could be elicited by other substances. However, the degree of change was greater with the blastomycetic polysaccharide.

Comment could be made on the microscopic character of the peritoneal exudates, the retrosternal nodes and the spleens. However, nothing additional to what has already been reported [11] concerning phagocytosis of polymorphonuclear neutrophils and peritoneal reaction was learned, and details are omitted.

It is apparent that these experimental results are very similar to those obtained from the injection of the tuberculo-polysaccharide as reported by Sabin, Joyner and Smithburn.[11] The increase in the immature polymorphonuclear cells and the decrease in the lymphocytes of the blood stream occur as strikingly after the injection of blastomycetic polysaccharide as they do after the injection of the polysaccharides which these authors employed.

Without the employment of numerous control polysaccharides derived from many sources it would be difficult to maintain that the polysaccharides so far employed have any highly specific effect. The control substances have been dissimilar with respect to molecular weight and solubility.

Of greatest general interest is the information, not new, but confirmed, that exceedingly minute quantities of various substances injected intraperitoneally produce not only the mild, acute peritonitis which might be expected but also significant changes elsewhere in the body, as in the retrosternal nodes, blood stream and spleen (and bone marrow also, according to Sabin, Joyner and Smithburn [11]).

Summary and Conclusions

A study was made of the effects on mice of intraperitoneal injections of living *Blastomyces dermatitidis,* killed suspensions of the same organism and a phosphatide fraction of this fungus; also a polysaccharide fraction was studied in rabbits. The following results and conclusions are recorded:

1. The mouse was preëminently suited to the experimental production of blastomycosis. The experimental disease was characterized by the continued response of polymorphonuclear neutrophils to the luxuriant growth of the fungus throughout the infected animal and by the necrosis of the blastomycotic masses. The lesions in mice consisted mainly of organisms, whereas the lesions in most infectious diseases consist mainly of the reacting cells of the hosts.

2. Repeated intraperitoneal injections of heat-killed *B. dermatitidis* were toxic for mice, and often lethal. This is thought to explain the final lethal effect in the experimental disease, in which masses of organisms and intermingled reacting cells became necrotic, and probably permitted the absorption of substances like those associated with the suspensions of the heat-killed organisms.

Heat-killed and living blastomycetes provoked similar cellular responses in the peritoneal cavities of mice.

3. Blastomycetic phosphatide repeatedly injected intraperitoneally into mice caused cells of the monocytic series to respond. This fraction is therefore not responsible for the polymorphonuclear response and the necrotizing effect related to the living organisms.

4. In rabbits, single intraperitoneal injections of blastomycetic polysaccharide produced, in the first few hours, sterile peritonitis, retrosternal lymphadenitis and remarkable changes in the blood stream consisting of leukopenia, lymphopenia and increase in the numbers of immature amphophils. These changes are similar to those which have been produced by polysaccharides from tubercle bacilli and pneumococci, as described by Sabin, Joyner and Smithburn.

REFERENCES

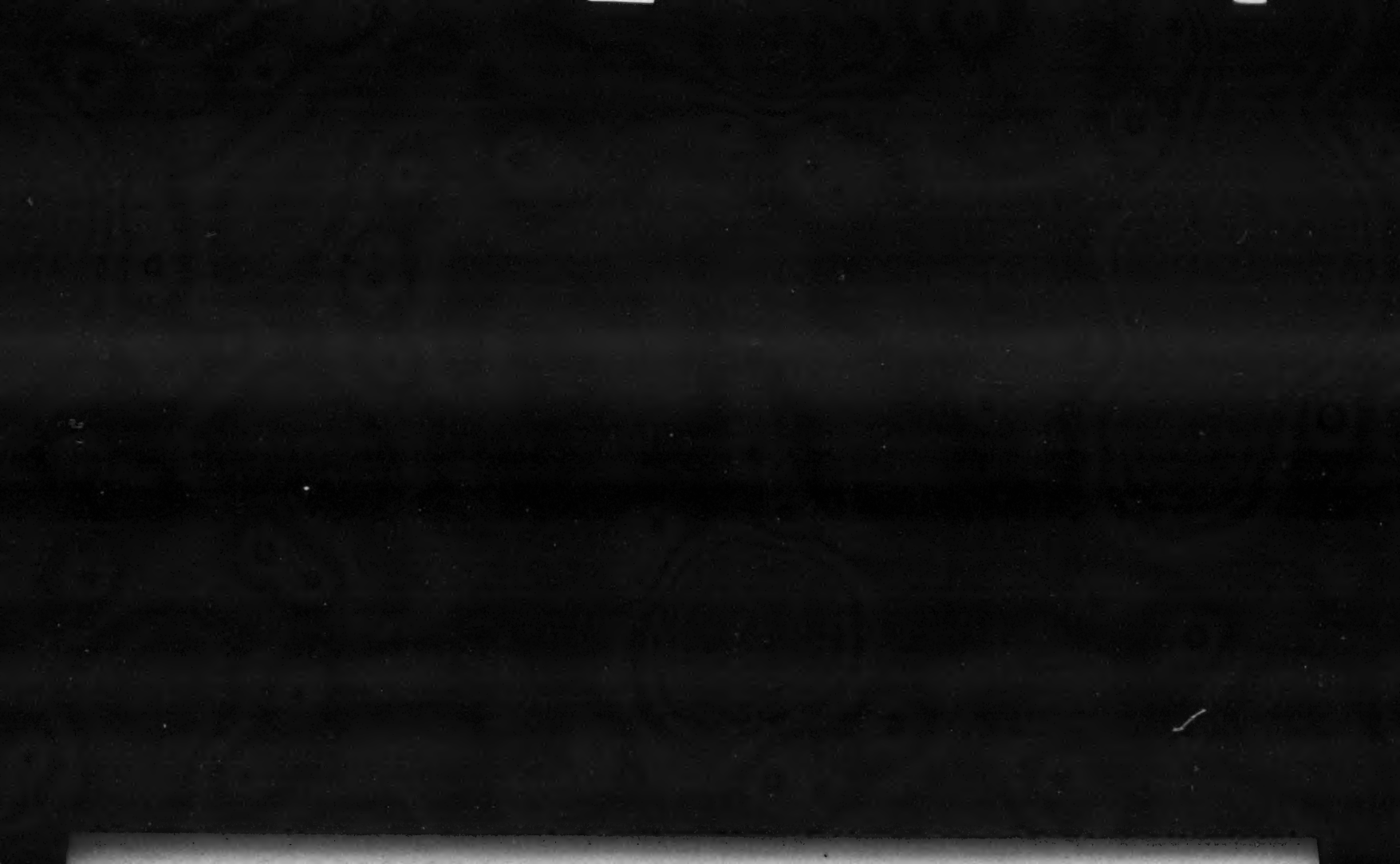
1. Baker, R. D. The effect of mouse passage on cultural characteristics and virulence for mice of organisms causing blastomycosis. *Am. J. Trop. Med.*, 1939, **19**, 547–563.
2. Baker, R. D. Comparison of infection of mice by mycelial and yeast forms of *Blastomyces dermatitidis*. *J. Infect. Dis.*, 1938, **63**, 324–329.
3. Peck, R. L., and Hauser, C. R. Chemical studies of certain pathogenic fungi. I. The lipids of *Blastomyces dermatitidis*. *J. Am. Chem. Soc.*, 1938, **60**, 2599–2603.
4. Peck, R. L.; Martin, D. S., and Hauser, C. R. Polysaccharides of *Blastomyces dermatitidis*. *J. Immunol.*, 1940, **38**, 449–455.
5. Martin, D. S., and Smith, D. T. Blastomycosis. I. A review of the literature. *Am. Rev. Tuberc.*, 1939, **39**, 275–304.
6. Bowen, J. T., and Wolbach, S. B. A case of blastomycosis: the results of culture and inoculation experiments. *J. Med. Research*, 1906, **15**, 167–177.
7. DeMonbreun, W. A. Experimental chronic cutaneous blastomycosis in monkeys. A study of the etiologic agent. *Arch. Dermat. & Syph.*, 1935, **31**, 831–854.
8. Bergstrom, V. M.; Nugent, Grace, and Snider, M. C. Blastomycosis. Report of a case with involvement of the skin and bones. *Arch. Dermat. & Syph.*, 1937, **36**, 70–76.
9. Davis, B. F. The immunological reactions of oidiomycosis (blastomycosis) in the guinea-pig. *J. Infect. Dis.*, 1911, **8**, 190–240.
10. Thomas, R. M., and Dessau, F. I. Experimental tuberculosis in mice. The cellular response to the chemical fractions of the tubercle bacillus. *Yale J. Biol. & Med.*, 1939–40, **12**, 185–198.
11. Sabin, F. R.; Joyner, A. L., and Smithburn, K. C. Cellular reactions to polysaccharides from tubercle bacilli and from pneumococci. *J. Exper. Med.*, 1938, **68**, 563–582.

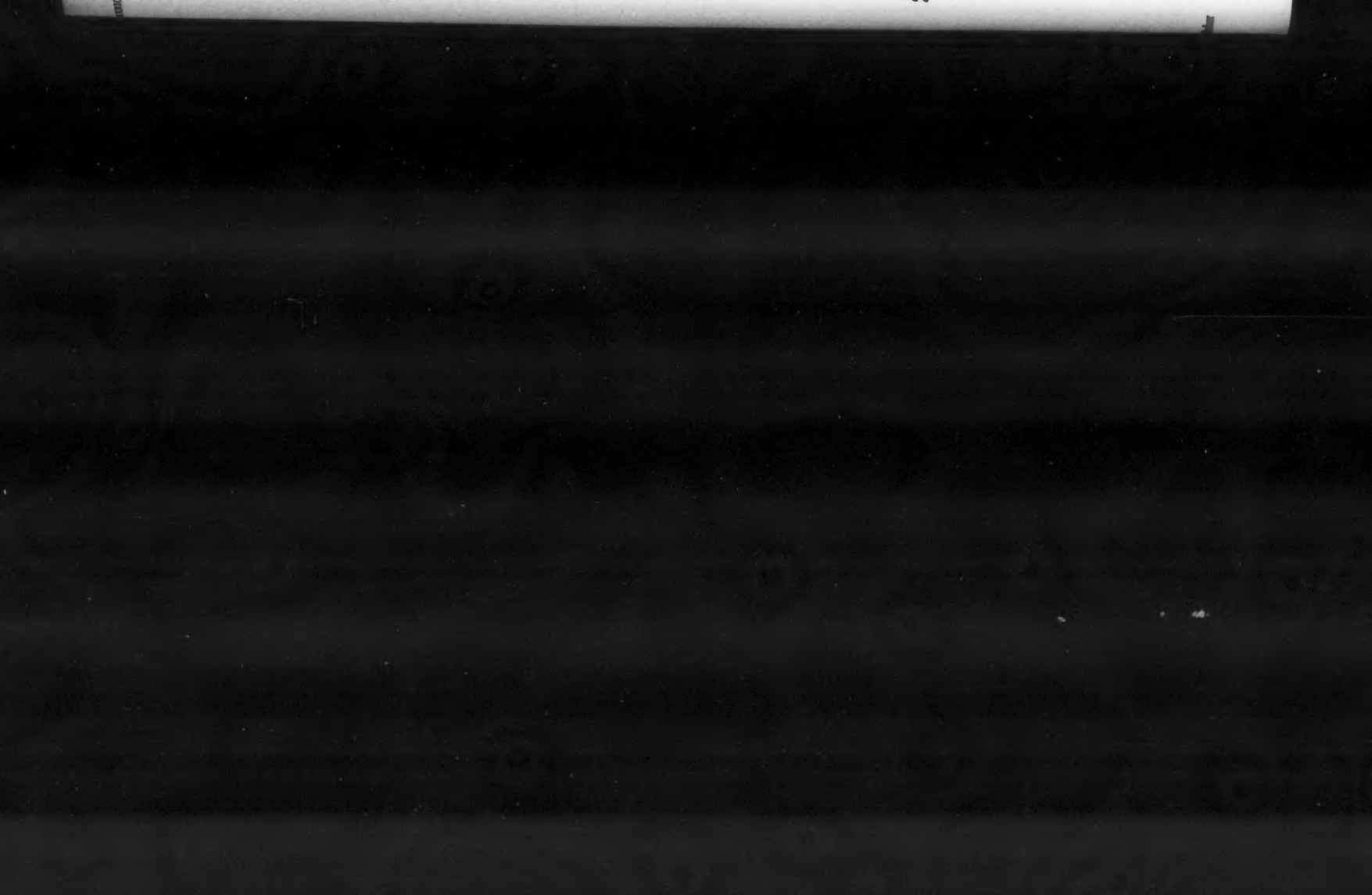
DESCRIPTION OF PLATES

PLATE 76

FIG. 1. Cellular reaction about living blastomycetes several hours after intraperitoneal injection. Peritoneal fluid of mouse. Neutral red, supravital preparation. × 715.

FIG. 2. Similar preparation, 8 days after injection. Two blastomycetes below in mass of reacting cells; fibroblasts above and to the right. × 715.





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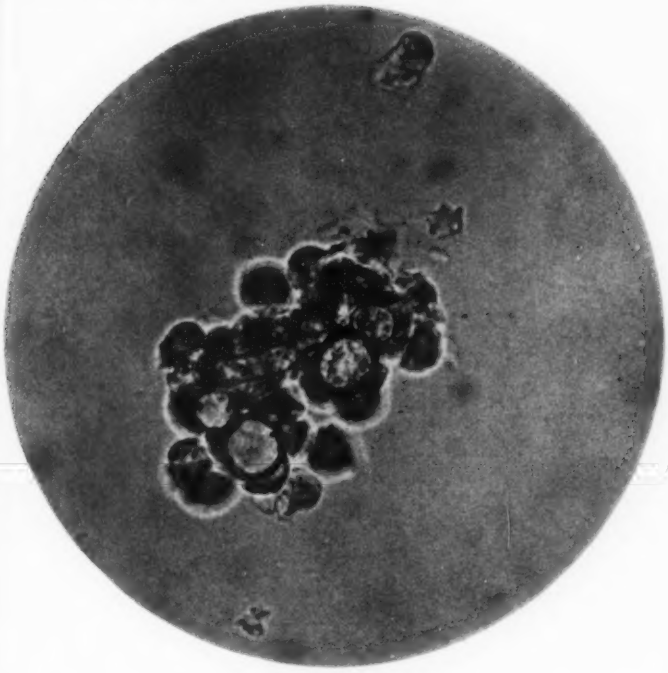

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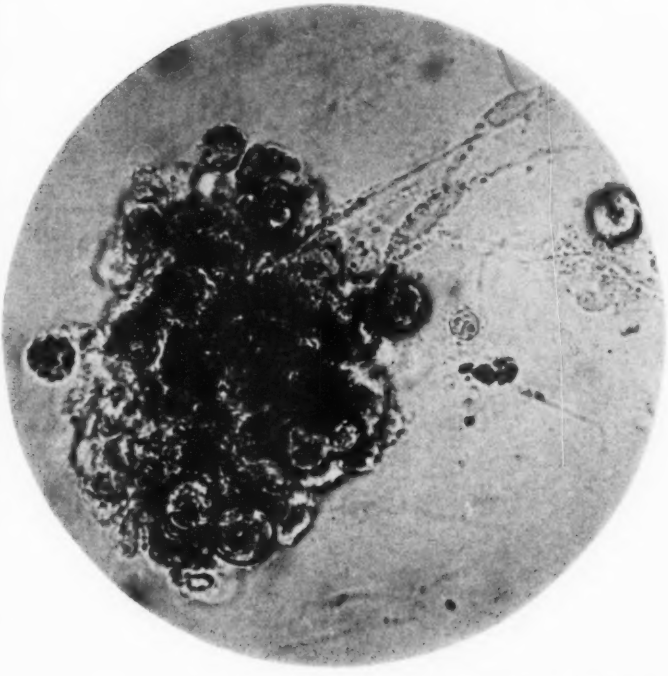


Experimental Blastomycosis in Mice

PLATE 77

FIG. 3. Necrosis in center of peritoneal blastomycotic mass. Experimental blastomycosis in a mouse, 5 days after intraperitoneal injection. × 174.

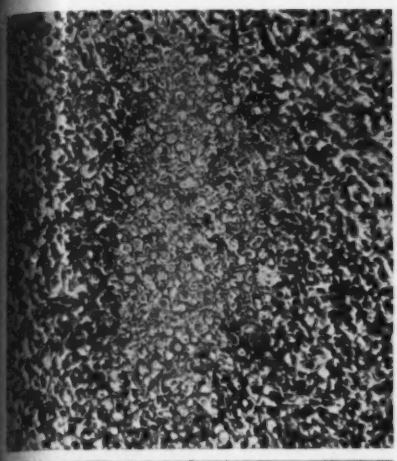
FIG. 4. Focal lesion in peritoneum; single blastomycete surrounded by polymorphonuclear neutrophils. Mouse, 11 days after injection. × 760.

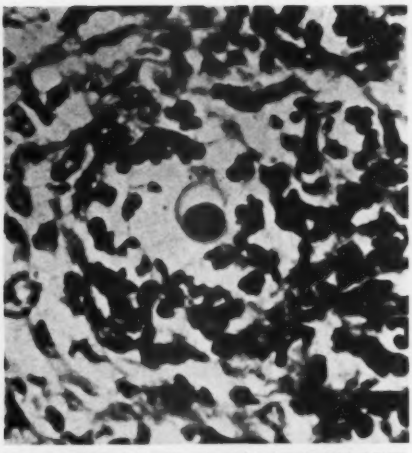
FIG. 5. Large pulmonary nodules in a mouse, 20 days after intraperitoneal injection. The pale, central areas are largely necrotic. × 14.

FIG. 6. Higher magnification of area in the preceding figure. This field is from the periphery of a pulmonary nodule where the blastomycetes are viable, as indicated by the staining of the internal substance of the blastomycete. The interstices between blastomycetes are filled with cells, chiefly polymorphonuclear neutrophils, and nuclear fragments. × 760.

FIG. 7. Cellular response in peritoneum to heat-killed blastomycetes, 5 days after initial intraperitoneal injection. There is a similarity to Figure 6, except for the lack of staining of the central portions of the blastomycetes, indicating that they are dead. × 760.

FIG. 8. Reaction to repeated injections of blastomycetic phosphatide. Cells of the monocytic series on the parietal peritoneum; fibroblasts are also present. This mouse received intraperitoneally 30 mg. of phosphatide in distilled water daily for 24 days, and was sacrificed 3 days after the last injection. × 760.





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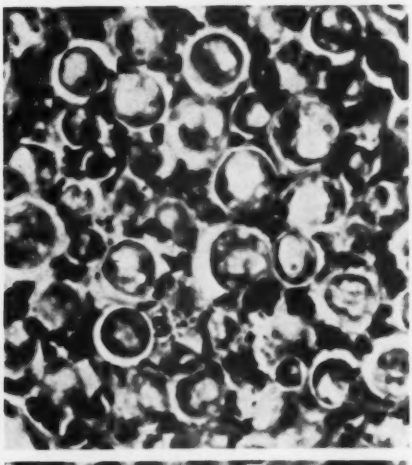

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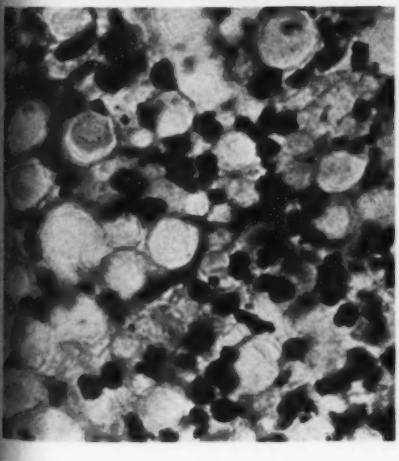

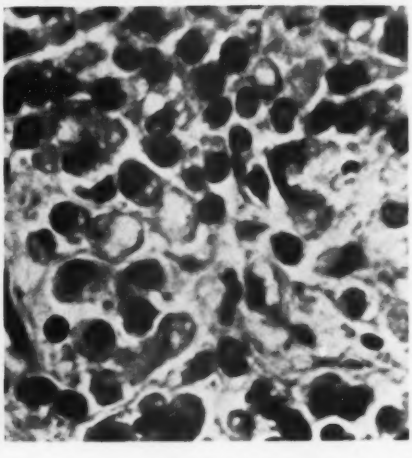

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Experimental Blastomycosis in Mice

TISSUE REACTIONS IN HUMAN BLASTOMYCOSIS *

An Analysis of Tissue from Twenty-Three Cases

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(From the Department of Pathology, Duke University School of Medicine, Durham, N. C.)

In North Carolina many human cases of blastomycosis have been encountered. Reports on them have dealt primarily with the general and immunologic features of the disease,[1] or with particular features such as laboratory diagnostic,[2] gynecologic,[3] cardiac,[4] cutaneous[5] and mycologic [6] considerations. From animal experimentation in connection with these human cases it has become apparent that the mouse is preeminently suited to the experimental production of blastomycosis.[7] The comparatively abundant histologic material available has permitted a restudy of the reaction of the tissues of man to this large fungous organism. Since it has been stated that blastomycosis and tuberculosis are practically identical histopathologically,[8,9] this aspect of the reaction has been approached with especial interest.

In a comprehensive analysis of blastomycosis, Stober,[10] who based his conclusions on a large group of especially well studied cases, stated: "The changes most characteristic of blastomycosis are the cutaneous ulcerations, the deep and superficial abscesses, and the often tubercle-like nodules in the viscera." The emphasis in this quotation is on ulceration and abscess formation, although it is indicated that tubercle-like nodules occur. Others have adopted about the same view.

In more recent years the emphasis in papers on blastomycosis appears to have shifted somewhat. One might be led to believe that the tissue response in blastomycosis is identical with that in tuberculosis. Medlar [8] suggested this view in a report of two cases of pulmonary blastomycosis. D'Aunoy and Beven,[9] in a report of 26 cases, 16 of which were cutaneous, went so far as to state that "only the presence of the specific organisms either active or dead, allowed making an histological differentiation from tuberculosis in any of the cases." I supported this opinion in a case report,[3] but have now altered my view.

The observations to be reported reëmphasize the importance of abscess formation in blastomycosis, while they do not deny the occurrence, in some cases, of lesions like those commonly seen in tuberculosis. In most instances in which a differentiation was to be made by biopsy,

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the histologic appearance was thought to be highly characteristic. The presence of microscopic abscesses, certainly not common in tuberculosis, stimulated search for blastomycetes. On the other hand, it is not desirable to make the definite histopathologic diagnosis of blastomycosis without demonstration of blastomycetes.

All available clinical and pathologic data were analyzed in 23 cases of human blastomycosis from which tissue had been obtained. These occurred in the 10-year period ending in July, 1940. Twenty had been studied clinically at Duke Hospital. In 3, tissue came from other hospitals in North Carolina. Several patients were seen at Duke Hospital during the same decade from whom no biopsy had been obtained. For valuable data upon 11 of the 23 cases the reader is referred to other papers.[1,3,4]

For the present study, data were recorded in relation to the time when the tissues were obtained for examination, and the following facts

TABLE I

Human Blastomycosis, 23 Cases

Ages: 5 to 70 years

Race: Colored, 16; White, 7

Sex: Male, 20; Female, 3

Tissue available:
- Autopsies, 4
- Bone resections, 4
- Deep tissue resections, 2
- Skin biopsies or resections, 13

Involvement:
- Generalized, 7 cases
- Thoracic, 3 cases
- Skin, 13 cases (single lesions, 9 cases; multiple lesions, 4 cases)

Duration before histologic examination: 6 weeks to 13 years

Organism cultured: 16 cases

Microscopic sections:
- Viable organisms: always present
- Dead organisms: often present
- Polymorphonuclear abscesses: always present
- Giant cells: always present
- Caseation: present in generalized cases and many "deep" cases; not noted in skin cases

and observations in each case were tabulated: Race, age, sex, first manifestations of the disease, clinical course, proved or inferred involvement of the body, duration of the disease up to the time of histologic examination and outcome. The gross and histologic appearances of the lesions were studied in the light of additional data: whether iodides had been administered just before tissue was obtained, the results of skin tests and complement-fixation tests and the degree of

toxicity of the patient. In histologic study a quantitative estimate was made of each of the following features: viable blastomycetes, dead blastomycetes, polymorphonuclear abscesses, giant cells and caseation.

Basis of Diagnosis

In 16 of the 23 cases, *Blastomyces dermatitidis* was recovered from lesions, and identified by cultural methods in the Department of Bacteriology. The mycologic and other criteria for identification in these cases are given by Conant and Martin.[6] The organism grew as a budding yeast at body temperature, and as a mold, showing mycelial threads and lateral conidia, at room temperature.

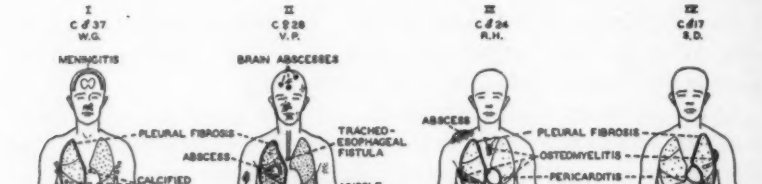
In all 23 cases double-contoured, yeastlike organisms, sometimes budding, were demonstrated in lesions regarded as characteristic of the disease. In my opinion, the histopathologic diagnosis of the disease, with the etiologic agent present in the lesions, is highly accurate—as accurate as the diagnosis of tuberculosis on the basis of finding acid-fast organisms in lesions characteristic of tuberculosis. Other yeastlike organisms, such as those of moniliasis, cryptococcosis (torulosis), or coccidioidal granuloma, are distinctive in sections. However, attempts should be made to obtain pure cultures in every case. Cultural methods of diagnosis, even without sections to show the characteristic tissue response, have proved to be reasonably accurate, since *B. dermatitidis* has not been shown to be a common secondary invader in human lesions or exudates. The method of direct examination of exudates or other material in 10 per cent sodium hydroxide should, of course, precede all other methods. In obscure pulmonary conditions in which blastomycosis is suspected, a skin test and complement-fixation reaction should be done.[11]

Involvement and Spread

The extent of involvement of the body was determined satisfactorily in the four cases upon which autopsies were done, as indicated in Text-Figure 1. In all four the lungs were involved. In cases 1, 2 and 3 there must have been hematogenous dissemination since lesions occurred throughout the body. In case 4 the lesions were confined to the thoracic region, and all could have extended directly from the oldest pulmonary lesion in the left lower lobe. This is the distinction between systemic and thoracic cases.

From the clinical histories it was thought that the portal of entry in the cases examined by autopsy was the respiratory tract. Therefore, thorough search was made for a primary pulmonary lesion such as occurs in tuberculosis, or for an old healed blastomycotic lesion which might have altered the susceptibility of the individual to reinfection.

An "oldest" lesion was identified in cases 2, 3 and 4. In case 2 this was a ragged abscess cavity in the right middle lobe (Fig. 8); in case 3, blastomycotic scarring of the upper portion of the right lower lobe; and in case 4, blastomycotic scarring at the base of the left lung. In case 1 calcified foci were found in the right lower lobe and in the draining hilar node, which were as likely to be tuberculous as blastomycotic,



Text-Figure 1. The location of lesions in four cases of blastomycosis studied at autopsy.

if not more so. No organisms were demonstrable in the calcified lesions. Dense pleural fibrosis occurred in each case on the side which appeared to be primarily affected.

Thus in the four cases coming to autopsy there was no close analogy to adult or reinfection tuberculosis. The process began in one portion of the lungs, developed there for a time, and then spread to the rest of the lungs and usually to the general circulation. No stage of massive mediastinal blastomycotic lymphadenitis occurred, analogous to childhood or first-infection tuberculosis.

Lymphatic spread was noted as from lesions on the extremities to axillary or inguinal nodes, and from lungs to peribronchial nodes. Hence the lymphatic system was not immune to blastomycosis. The thoracic duct appeared to be normal, grossly and microscopically, in three cases. This does not prove that transmission of organisms cannot occur by this duct, but suggests that lymphangitis is not common in blastomycosis, in contrast to sporotrichosis.

Direct extension was a frequent and important method of spread:

from bone to skin surfaces, from spine to iliopsoas abscesses, from lung to pleura, and probably from pleura to pericardium. In case 2 a tracheo-esophageal fistula had formed in connection with a blastomycotic lymph node which lay between these channels.

The cases from which specimens of bone or deep tissues had been obtained were also systemic or thoracic. There was no evidence for primary bone involvement as from penetrating wounds.

In the cutaneous group, those examples with multiple ulcers widely separated on the body and those with roentgenographic evidence of "fibrosis of the lungs" suggested the possibility that some cutaneous lesions might have originated hematogenously, perhaps from a subclinical pulmonary focus. The only alternative explanation for widely separated skin lesions is auto-inoculation.

Most of the cases with cutaneous involvement were thought to be primary, probably from direct inoculation, as in wound infection, although precise information on this point was unobtainable. Cases are on record of cutaneous blastomycosis following traumatic injuries,[12] and cutaneous blastomycosis can be produced in monkeys by intracutaneous injections.[13] In most of our cases spread from single cutaneous lesions appeared to be by peripheral extension, either direct or by the cutaneous lymphatics. The cutaneous ulcerations found in the patients of autopsies no. 1 and 2 seemed to be of hematogenous origin.

Toxicity

A close correlation between toxicity and systemic or deep involvement was noted. Most of the patients with cutaneous lesions were ambulatory and "non-toxic." All of those who came to autopsy were judged to be 4 plus (++++) in respect to toxicity, on the basis of temperature, blood-cell studies and weakness. In three, death appeared to be due directly to blastomycotic involvement; in one, to heart failure secondary to constrictive blastomycotic pericarditis (case 4). An element of heart failure may have been present in case 3 also, and on the same basis.[4]

Study of the tissues suggested that the toxicity of the patient was related to two features: (1) large numbers of organisms and (2) caseation. In material obtained by autopsy and from deep lesions, organisms were often present in enormous masses, almost in pure culture, as in the brain abscess (Figs. 2 and 9) of autopsy no. 2. Organisms were often dead in the central portions of such masses, reminiscent of the findings in experimental blastomycosis in mice. In the cutaneous cases, on the other hand, organisms occurred singly or in pairs (Fig. 1) and the lesion was composed of cellular (polymorphonuclear) exudate, or granulation tissue and hyperplastic epithelium.

Duration and Outcome

The patients who came to autopsy had the disease for periods of between 3 and 24 months, so far as could be judged from their histories and physical evidence. Of the patients who had bone lesions, two died and two were living, 2 and 4 years after onset, without evidence of blastomycosis. One, a child of 5 years, who had osteomyelitis of a rib and miliary pulmonary involvement, revealed by x-ray examination, became free from evidence of the disease without iodides or other forms of therapy except drainage of the focus of osteomyelitis. Another patient was cured by a combination of therapeutic procedures.[14]

Of the two patients with resections of deep tissues, one is living and apparently free of blastomycosis 7 years after the onset of symptoms[3] (Martin and Smith,[1] case no. 10). The other patient with thoracic involvement and a supraclavicular subcutaneous abscess, cannot be traced.

Of the 13 patients with cutaneous involvement, two died 4 and 5 years after the onset, apparently of blastomycosis. Two showed no blastomycosis 2 and 5 years after onset. Most of the cutaneous cases have remained such without generalization, and have improved or have been cured by various forms of therapy. One has had a duration of 13 years, with involvement of the skin of the entire thorax and neck, but without impairment of general health.

Fundamental Gross Characteristics

The pyogenic character of the disease was usually impressive. Three of the patients coming to autopsy presented massive, fluctuating, subcutaneous abscesses (Text-Fig. 1) and all of them had osteomyelitis with sinuses extending to the skin or to joint surfaces. Brain, psoas and intramuscular (Figs. 3 and 4) abscesses were noted. The pus was usually pink. In a patient with a deep lesion, subjected to laparotomy, 25 cc. of creamy pus came from the tubes and pelvic peritoneal recesses.[3] In the cutaneous cases pus could be obtained from miliary abscesses at the periphery of the lesions.

Grossly the appearance of caseation was sometimes noted. In autopsy no. 2, the cut surface of the lung showed "large confluent areas of caseation from which pus can be squeezed." In autopsy no. 1, the fresh lungs at the time of autopsy showed "scattered, small yellow abscesses." After fixation, the same foci were spoken of as tubercles. In autopsy no. 3 the term "tubercles" was used for the pulmonary nodules, but it was noted that central softening was more apparent than in tuberculosis.

Fundamental Microscopic Characteristics

In every case the polymorphonuclear neutrophil was a prominent cell type (Figs. 1 to 5) and usually it occurred in abscesses. In two cases with deep lesions, true abscesses were not present and the polymorphonuclear reaction was spoken of as diffuse. Sometimes the polymorphonuclear neutrophils occurred in the interstices between blastomycetes, as in the massive lesions of the generalized type; and sometimes the opposite relationship existed, *i.e.*, one or two blastomycetes occurred in a mass of pus, as in the sparser involvement of the cutaneous cases. The former type of involvement is well illustrated by Mallory.[15] Eosinophils were occasionally prominent. Giant cells (Fig. 5) were noted in every case, but were sometimes very rare. Usually blastomycetes, either living or dead, occurred in giant cells, but in a few instances no organisms were noted. Large mononuclear cells were not prominent.

Caseation was not found in the lesions of the solely cutaneous cases, nor in the skin lesions of the cases coming to autopsy. In fact, the skin lesions of the latter group closely resembled those of the "pure" skin cases. Abundant caseation was noted in autopsies nos. 1, 3 (Fig. 6) and 4, and in all the cases with osseous and deep lesions except two. In specimens obtained at autopsy it could sometimes be determined, from the presence of shadows of dead blastomycetes, that caseous material was composed largely of dead fungi. This was true of lesions of the heart, brain, prostate and bone. In some of the caseous pulmonary lesions, on the other hand, no shadows were noted in the caseous material. It was not evident whether the necrosis was due to proteolytic enzymes of the contained polymorphonuclear neutrophils, or to the poor nutrition of those blastomycetes which lay centrally, or to other factors.

Fibrosis was frequently prominent (Fig. 7). In many lesions fibrosis about abscesses had developed a heavy collagenous component.

Viable organisms were always present, free or within giant cells. Dead organisms, free or within giant cells, were present in material obtained by autopsy, and in most of the cutaneous lesions. The differentiation between viable and dead organisms was made on the basis of staining reaction. Blastomycetes appearing as shadows were obviously dead. Forms in which the central portion of the organism no longer absorbed any hematoxylin were also considered dead. The validity of this differentiation was supported by the appearance, in sections, of the heat-killed blastomycetes injected experimentally into mice.

From this analysis, it is clear that the microscopic characteristics of blastomycosis are not identical with those of tuberculosis. In the tabu-

lation, similarity to tuberculosis was designated 1 plus (+) in seven cases and 2 plus (++) in one case. But even in these cases, other areas deviated from the characteristics of tuberculosis.

Iodides

An attempt was made to correlate the histologic picture and the number of living or dead organisms with the administration of iodides. No clear-cut conclusions could be adduced. In four of the cases massive doses had been given shortly before histologic examination. Two of these were cases examined at autopsy. Comparison with the other two similarly examined, in which iodides had not been given, showed no essential differences with respect to the organisms, the degree of caseation, or the fibrosis. In a surviving systemic case [3] in which laparotomy had been performed, desensitization and iodide therapy were carried out according to the method of Martin and Smith.[11] In the pelvic material there were blastomycotic tubercles with more fibrosis about them than in any other case (Fig. 7). In the same region, however, there were other areas with pus and organisms, and with all stages of inflammatory reaction and repair. (The reader is referred to the illustrations in the paper by Hamblen, Baker and Martin.[3]) In a cutaneous case, healing of lesions with dense fibrosis, devoid of blastomycetes, occurred. Iodides and desensitization had been used, but x-ray treatment had also been given. Thus the evidence as to the effect of iodides on the histologic picture of blastomycosis is not clear; possibly fibrosis is stimulated. Clinically, the administration of potassium iodides by mouth is said to promote marked improvement in the majority of cutaneous cases, but in the systemic cases the results are very discouraging.[11]

It has not been possible to evaluate histologically the effect of x-ray treatment in this series, since there were not enough biopsies after such treatment. Clinically the cutaneous lesions appear to undergo regression and fibrosis with x-ray treatment,[16] but usually too many factors have been present in the therapy to evaluate any one of them properly.

Immunologic and Allergic Responses

The pathologic analysis was made with the following observations of Martin and Smith [1] in mind:

"Antibodies can be found in the sera of patients who are heavily infected; they persist until death unless the infectious process is overcome or greatly reduced.

"Some patients develop a condition of hypersensitiveness to the infecting fungus, and this allergic state diminishes in the terminal stages of the disease."

A correlation of histopathologic details with such clinical observations could be made only in so far as these details were associated with

either severe systemic infection or with mild, localized cutaneous infection. When antibodies were high or when the patient was in the terminal anergic state, great numbers of organisms were present and caseous necrosis was often noted.

Discussion

In affirming the characteristic nature of the tissue response in blastomycosis, I am impelled to comment on the opposite point of view as expressed by Medlar.[8] He emphasized the identity of the gross and microscopic pathology in fungous and tuberculous infections in connection with a report of two cases of pulmonary blastomycosis. This statement seems to me to be far too sweeping even if the problem is approached as Medlar approached it, by comparison of the blastomycotic reaction with the histogenesis of tuberculosis, with special regard to the polymorphonuclear neutrophil, caseation and the presence of reticulum. In generalizing about blastomycosis, moreover, he used case reports of pulmonary blastomycosis alone upon which to base his opinion. It has been shown above that these are the very cases which may resemble tuberculosis most strongly.

Abscess, a circumscribed collection of pus, with the connotation of a liquid state in the unfixed condition at body temperature, may occur in tuberculosis, it is true. But if 23 cases of tuberculosis were accepted *seriatim*, the observer would certainly be far less impressed by the presence of abscesses grossly and microscopically than in the 23 cases of blastomycosis here reported.

The reverse of this would be true with respect to caseation. The term caseation is applied to the gross characteristics of the necrotic material commonly seen in tuberculous infection. If this term is applied to the necrotic material commonly seen in blastomycotic infection, in some instances the microscopic character of caseation in the two diseases will correspond closely. But in other instances, as has been pointed out, blastomycotic caseation may consist largely of masses of dead blastomycetes and not of reacting cells of the host. In tuberculosis this situation is encountered only in those very rare instances in which masses of stained tubercle bacilli can be perceived in a histologic section with the naked eye.

Miller,[17] using Bielschowsky's silver method (which is excellent to demonstrate blastomycetes and reticulum), showed that the growth and transformation of collagenous tissue differed in no way in the tubercle of blastomycosis from that in the tubercle of tuberculosis. While this is a point of resemblance between the reactions of the two diseases, it should be remembered that the production of reticulum

and collagen is a general pathologic process related to a stage in repair, and not peculiarly associated with these diseases, nor with all stages of these diseases.

Summary and Conclusions

1. Among 23 cases of human blastomycosis (13 cutaneous and 10 generalized or thoracic) complete autopsy was available in 4, and histologic material in the remaining.

2. The formation of abscesses was an impressive gross feature of the generalized cases, and polymorphonuclear foci were noted microscopically in all 23 cases. Giant cells were always present. Caseation was present in the generalized and thoracic cases, but was not noted in the cutaneous cases.

3. Human blastomycosis was interpreted as being primarily pyogenic, with prominence of polymorphonuclear neutrophils. Some lesions of some cases, especially in the systemic group, closely resembled the lesions of tuberculosis.

4. The terminal stage of systemic blastomycosis in man corresponded closely to the experimental disease in the mouse. Masses of blastomycetes occurred with necrosis, producing a toxic effect upon the patient. In the cutaneous cases, in contrast, organisms were usually moderate in numbers, caseous necrosis was usually absent and the lesion was composed of miliary abscesses, granulation tissue and hyperplastic epidermis. There was little toxic effect upon the patients.

5. These observations on the nature of toxicity in blastomycosis suggest that therapeutically a fungicide is not desirable in the severe systemic cases, since too much necrotic blastomycotic material is already present.

REFERENCES

1. Martin, D. S., and Smith, D. T. Blastomycosis. II. A report of thirteen new cases. *Am. Rev. Tuberc.*, 1939, **39**, 488–515.
2. Martin, D. S., and Smith, D. T. The laboratory diagnosis of blastomycosis. *J. Lab. & Clin. Med.*, 1935–36, **21**, 1289–1296.
3. Hamblen, E. C.; Baker, R. D., and Martin, D. S. Blastomycosis of the female reproductive tract. With report of a case. *Am. J. Obst. & Gynec.*, 1935, **30**, 345–356.
4. Baker, R. D., and Brian, E. W. Blastomycosis of the heart. Report of two cases. *Am. J. Path.*, 1937, **13**, 139–147.
5. Callaway, J. L., and Moseley, Vince. Primary cutaneous Gilchrist's disease. *South. M. J.*, 1940, **33**, 622–626.
6. Conant, N. F., and Martin, D. S. A comparative study of nineteen strains of *Blastomyces dermatitidis*, Gilchrist and Stokes, 1898. (To be published.)
7. Baker, R. D. Experimental blastomycosis in mice. *Am. J. Path.*, 1942, **18**, 463–477.

8. Medlar, E. M. Pulmonary blastomycosis; its similarity to tuberculosis. Report of two cases. *Am. J. Path.*, 1927, **3**, 305–314.
9. D'Aunoy, Rigney, and Beven, J. L. Systemic blastomycosis. *J. Lab. & Clin. Med.*, 1930–31, **16**, 124–130.
10. Stober, A. M. Systemic blastomycosis. A report of its pathological, bacteriological and clinical features. *Arch. Int. Med.*, 1914, **13**, 509–556.
11. Martin, D. S., and Smith, D. T. Blastomycosis. I. A review of the literature. *Am. Rev. Tuberc.*, 1939, **39**, 275–304.
12. Cole, W. H. Systemic blastomycosis (oidiomycosis). *Ann. Surg.*, 1924, **80**, 124–134.
13. DeMonbreun, W. A. Experimental chronic cutaneous blastomycosis in monkeys. A study of the etiologic agent. *Arch. Dermat. & Syph.*, 1935, **31**, 831–854.
14. Martin, D. S., and Jones, R. R., Jr. Systemic blastomycosis. Report of a case with unusual immunologic findings, well two years after onset. *Surgery*, 1941, **10**, 939–946.
15. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Company, Philadelphia, 1925.
16. Reeves, R. J. Personal communication.
17. Miller, W. S. The reticulum of the lung. V. Its similarity in blastomycosis to that in tuberculosis. *Am. J. Path.*, 1927, **3**, 315–320.

DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Microscopic abscess in cutaneous blastomycosis. Blastomycetes, one budding, are free in the center. There is a predominantly polymorphonuclear response, but other types of cells are present. The epidermis appears below. From skin of nose of white man, 63 years old, with multiple cutaneous lesions. Biopsy was taken 3 years after onset of disease. The patient was living in 1938, 2 years later, without evidence of blastomycosis. × 705.

FIG. 2. Massive growth of blastomycetes in an abscess of the human brain, autopsy no. 2. (Photograph of the gross specimen is shown in Fig. 9.) Viable blastomycetes, from the edge of the abscess, are above; and necrosis, toward the center of the abscess, is below and to the left. × 705.

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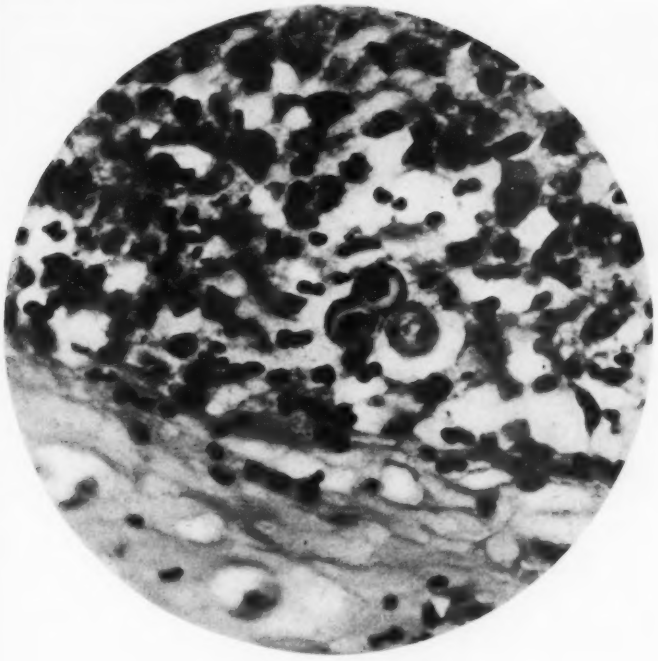

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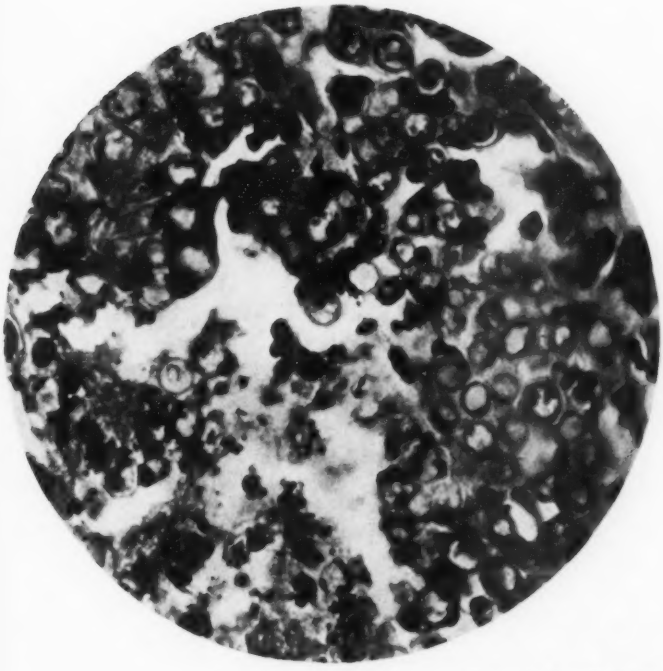


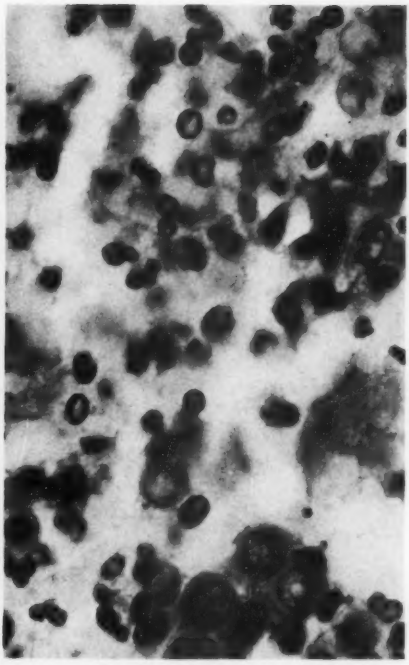
Experimental Blastomycosis in Mice

PLATE 79

FIG. 3. Wall of an abscess, and exudate, in muscle of upper arm, autopsy no. 2. × 174.

FIG. 4. Higher power magnification of area in Figure 3 to show free blastomycetes with cellular response rich in polymorphonuclear cells. Mononuclear cells are also numerous. × 760.

FIG. 5. Abscess and giant cells containing blastomycetes in an osteomyelitic case. This was a surgical specimen. Osteomyelitis of elbow and rib in a white male, 30 years old. The patient was apparently cured by a variety of therapeutic methods and was well 2 years after onset. (See report of Martin and Jones.[14]) × 174.



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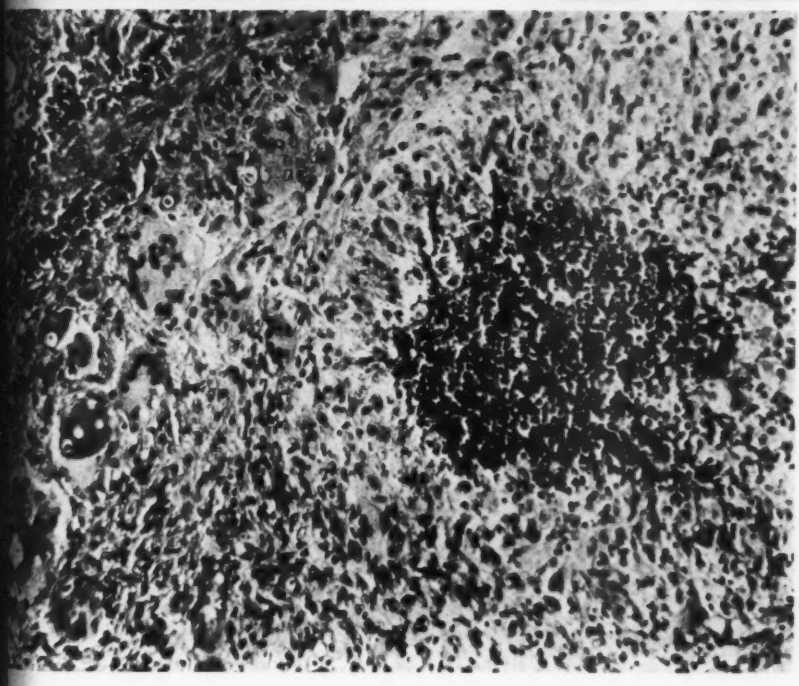

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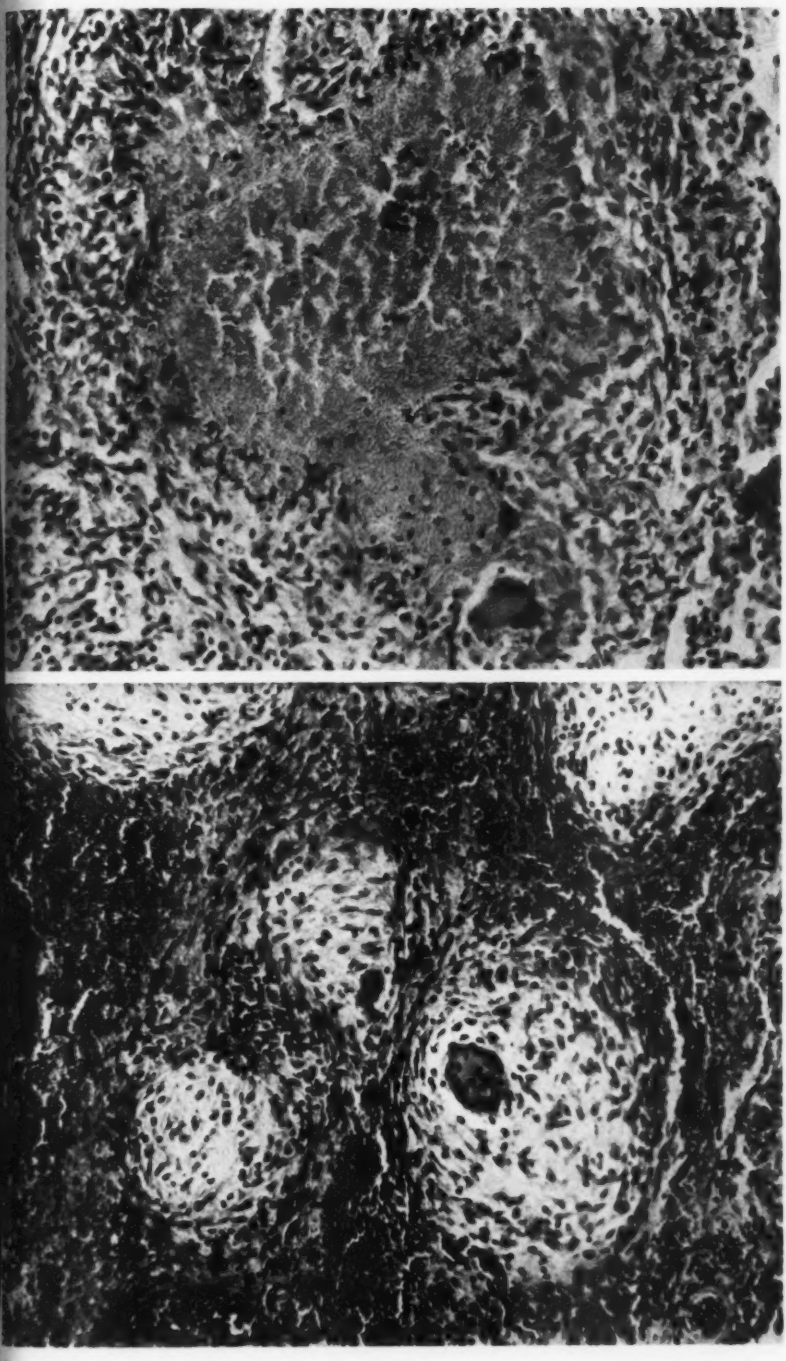
Experimental Blastomycosis in Mice

PLATE 80

FIG. 6. Caseous pulmonary lesion in autopsy no. 3, upon a case of generalized blastomycosis. Shadows of necrotic blastomycetes were seen in several levels of this lesion and in the adjacent giant cells. Acid-fast bacilli were not found in sections appropriately stained. × 174.

FIG. 7. Hard tubercles in peritoneal reaction about blastomycosis of fallopian tubes. Surgical specimen from white woman, 27 years of age, with generalized blastomycosis, now (1941) apparently free of the disease, 7 years after onset.[3] Blastomycetes were present in adjacent tubercles. Acid-fast bacilli were not found. × 174.

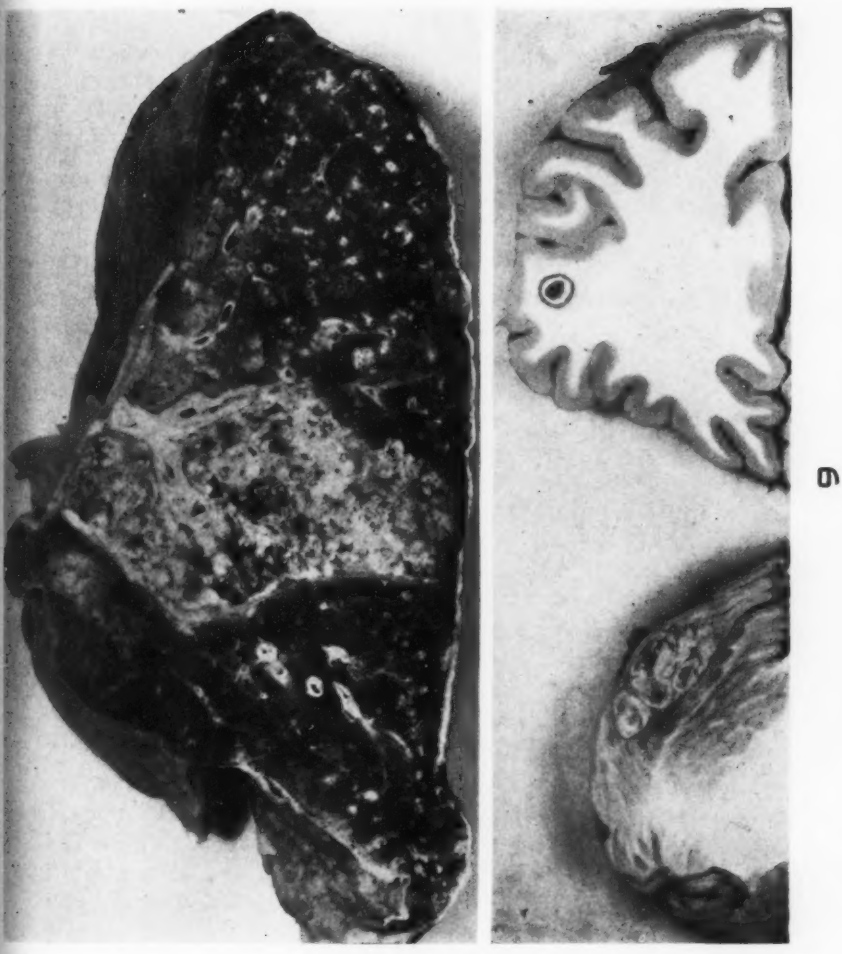


Experimental Blastomycosis in Mice

PLATE 81

FIG. 8. Blastomycotic pulmonary abscess (autopsy no. 2).

FIG. 9. Blastomycotic brain abscesses (autopsy no. 2).



Experimental Blastomycosis in Mice

PATHOLOGY AND PATHOLOGIC DIAGNOSIS OF RADIATION LESIONS IN THE GASTRO-INTESTINAL TRACT *

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The occurrence of lesions in the stomach and intestine as a consequence of radiation therapy is well known. Their nature, frequency and pathogenesis are dealt with in a current review [1] and in other publications,[2] so that material will not be repeated here. The purpose of the present paper is to review the radiation reactions of the gastro-intestinal tract studied in this laboratory and to present the pathologic findings in detail. It is hoped to emphasize those features which may be useful as diagnostic criteria of radiation reaction. With the increasing use of radiation therapy in high doses, lesions are being frequently encountered with which pathologists should be familiar. A determination on pathologic grounds whether a given lesion is entirely or in part due to radiation may have importance for further therapy.

The 38 cases employed for study were selected from a considerably larger group in our files. The cases selected were those in which the lesions showed a marked radiation reaction and in which the details of radiotherapy were available. An outline summary of these cases is presented (Table I).

The lesions may be grouped (Table II) according to their location in the gastro-intestinal tract and the nature of the reaction. They include ulcers, fistulae and strictures. A reaction comprising fibrosis and scarring, with thickening and induration of the bowel wall in the absence of ulceration, has been termed "sclerosis." Sclerosis alone may be marked enough to cause a stricture. Many reactions combined ulceration and stricture formation.

Many of the lesions developed at sites distant from neoplastic tissue but some occurred at the site of a partially or completely destroyed tumor and must be considered as "mixed" lesions. For example, in the bases of some ulcers exhibiting radiation reaction there was widespread infiltration of neoplastic tissue. However, since the radiation in most cases had been directed at a malignant process, many of the radiation lesions were in proximity to neoplastic tissue although they themselves were free of tumor. For example, in one case rectal ulceration characteristic of radiation reaction was present, while the

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† Littauer Fellow in Pathology.

TABLE I

Summary of 38 Cases Showing Radiation Lesions of the Gastro-Intestinal Tract

Primary disease	Radiotherapy		Duration of treatment (if multiple courses)	Interval from completion of treatment to time of pathologic examination	Method by which tissue was obtained	Radiation lesion		Neoplastic infiltration as part of lesion
	Roentgens	Milligram or millicurie hours				Site	Nature	
1. Tumor of stomach	5,400	.....	.....	1 week	Resection	Stomach	Ulcer with chronic perforation	Nodules of necrotic and unidentifiable tumor in base of ulcer; leiomyoma (?)
2. Generalized giant-follicular lymphoma	3,000	.....	17 months	2 months	Necropsy	Stomach	Ulcer	Probable regressed lymphoma at site
3. Carcinoma of cervix recurrent following panhysterectomy	7,200	.....	.....	7 months	Necropsy	Ileum (adherent in pelvis)	Ileo-vaginal fistula	Present
4. Carcinoma of rectum	8,900	.....	26 months	3 months	Necropsy	Small intestine (adherent to retroperitoneal metastases)	Ulcer	Present
5. Carcinoma of retained cervical stump	.....	3,500	.....	1 month	Resection	Ileum (adherent to cervical stump)	Sclerosis with stenosis	None
6. Carcinoma of cervix	.....	4,700	.....	7 years	Resection	Ileum (fresh adhesions to broad ligament; laparotomy 1 year earlier for gastro-intestinal symptoms and not adherent then)	Multiple (2) ulcers with stenosis	None

7. Carcinoma of cervix	.....	4,900	.....	7 years	Resection	Ileum (adherent to pelvic brim)	Multiple (2) ulcers with stenosis	None
8. Carcinoma of cervix	6,000	4,000	.....	8 months	Resection	Ileum (adherent to pelvic brim)	Multiple (5) ulcers with stenosis	None
9. Carcinoma of cervix	13,000	3,500	17 months	7 months	Resection	Small intestine (free loop)	Ulcer with stenosis	None
10. Carcinoma of retained cervical stump	.....	3,500	.....	3 months	Resection	Small intestine (adherent to cervical stump)	Entero-cervical fistula	None
11. Carcinoma of cervix	7,600	3,400	3 months	2 months	Necropsy	Ileum (adherent to uterus)	Ileo-uterine fistula	None
12. Carcinoma of rectum	1,500	7,800	.....	3 months	Necropsy	Jejunum (adherent to rectum)	Ulcer	None
13. Generalized Hodgkin's disease	1,900 and additional treatment dose unknown	.....	4 years	1 month	Necropsy	Small intestine	Multiple ulcers with perforation	Probable regressed Hodgkin's tissue at site
14. Ewing's tumor of ilium	3,500	.....	4 months	Immediate	Necropsy	Appendix	Sclerosis	None
15. Carcinoma of uterus	.....	6,700	2½ years	4 months	Necropsy	Sigmoid	Sclerosis	None
16. Carcinoma of rectum	.....	2,500	2 months	5 months	Resection	Rectum	Ulcer with stenosis	None
17. Carcinoma of cervix	.....	5,500	.....	11 months	Necropsy	Rectum	Sclerosis	None
18. Carcinoma of anus, recurrent following resection	.....	16,500	5 months	6 months	Necropsy	Rectum	Sclerosis	None
19. Carcinoma of cervix	.....	7,000	4 years	20 months	Necropsy	Sigmoid	Sigmoido-uterine fistula	None
20. Carcinoma of cervix	3,500	5,200	7 months	1 month	Necropsy	Rectum	Recto-uterine fistula	None
21. Carcinoma of anus	.....	900	.....	7 months	Resection	Rectum	Ulcer	None
22. Carcinoma of cervix	6,400	3,000	3 months	5 months	Necropsy	Sigmoid	Ulcer	None
23. Carcinoma of rectum	2,900	4,500	3 months	4 months	Resection	Rectum	Ulcer	None

TABLE I—*Continued*

Primary disease	Radiotherapy		Duration of treatment (if multiple courses)	Interval from completion of treatment to time of pathologic examination	Method by which tissue was obtained	Radiation lesion		Neoplastic infiltration as part of lesion
	Roentgens	Milligram or millicurie hours				Site	Nature	
24. Carcinoma of vagina	.....	8,600	.....	4 months	Resection	Rectum	Recto-vaginal fistula	None
25. Carcinoma of rectum	.....	1,600	.....	9 months	Resection	Rectum	Sclerosis	None
26. Carcinoma of rectum recurrent following local resection	.....	3,000	5 months	8 months	Resection	Rectum	Ulcer	None
27. Carcinoma of rectum	6,000	3,800	8 months	8 months	Resection	Rectum	Ulcer	None
28. Carcinoma of cervix	7,200	2,800	.....	5 months	Biopsy	Rectum	Ulcer with stenosis	None
29. Carcinoma of cervix	.....	2,800	.....	3 months	Necropsy	Rectum	Ulcer	None
30. Carcinoma of rectum	10,000	.....	5 months	9 months	Necropsy	Rectum	Sclerosis	None
31. Carcinoma of cervix	4,800	8,100	4¾ years	2 months	Necropsy	Rectum	Recto-cervical fistula	None
32. Carcinoma of cervix	4,000	3,000	2 months	10 months	Necropsy	Rectum	Sclerosis	None
33. Carcinoma of cervix	1,800	6,900	6 months	2 months	Necropsy	Rectum	Recto-vaginal fistula	Present
34. Carcinoma of rectum	2,800	3,600	2 months	2 weeks	Necropsy	Rectum	Ulcer	Present
35. Carcinoma of rectum	.....	2,600	14 months	6 months	Resection	Rectum	Ulcer	Present
37. Carcinoma of rectum	6,300	.....	.....	2 months	Necropsy	Rectum	Ulcer	Present
36. Carcinoma of rectum	.....	1,700	4 months	9 months	Resection	Rectum	Ulcer	Present
38. Carcinoma of rectum	8,400	.....	.....	1 month	Resection	Rectum	Sclerosis	Present

perirectal tissues were extensively invaded by carcinoma that arose in the cervix. In another case carcinoma of the rectum had undergone extensive necrosis following radiotherapy, while the nearby sigmoid showed typical radiation sclerosis. It is felt that such lesions should be considered as radiation reactions independent of the neoplastic process.

TABLE II

Distribution of the Lesions in the Gastro-Intestinal Tract and Their Relationship to the Presence of Neoplasm

	Radiation reaction without tumor			Radiation reaction with tumor		
	Ulcer or fistula	Ulcer with stenosis	Sclerosis or stenosis	Ulcer or fistula	Ulcer with stenosis	Sclerosis or stenosis
Large intestine	10	1	6	5	1	1
Small intestine	3	4	1	3	..	..
Stomach	..	..	..	2	..	..
Appendix	..	..	1	..	..	..

PATHOLOGIC ANATOMY

The lesions consisted of ulceration, sclerosis and combinations of the two. The process sometimes involved a segment of intestine uniformly; in other instances it was focal, with single or multiple lesions.

The bowel wall was usually thickened and indurated, with the serosa opaque and showing prominent telangiectasia. The mesentery, particularly at its point of attachment, was frequently similarly involved. The mucosa rarely appeared entirely normal, but usually, even in non-ulcerated lesions, atrophy and fixation to the submucosa were seen. The degree of ulceration ranged from confluent, irregular, superficial erosions to deep, punched-out ulcers. The persisting mucosa was irregularly heaped up and nodular in the former, while discrete ulcers were very sharp-edged. Telangiectasia occurred especially at the edges of ulcerated areas. Stenosis was sometimes due to diffuse sclerosis with general constriction of a segment, and sometimes to formation of a stricture at a site of ulceration.

Secondary changes, such as inflammatory reactions related to penetrating or perforating ulcers and fistulae, were evident. Exudation of fibrin, fibrinopurulent membranes and adhesions were encountered. Necrosis was a part of most reactions and in extreme instances the reaction approached massive gangrene of a loop of intestine.

showing a distinct, glassy, homogeneous, acidophilic structure similar to that produced by a physical agent such as the heat of a cautery.

The inflammatory reactions were quite varied and all types of cellular response were seen. Granulation tissue frequently formed in the usual manner although occasionally the degenerated hyalinized tissues remained almost avascular.

Diagnosis

Knowledge of the changes seen in radiation reactions is of value from two points of view. On the one hand these changes are of academic and biologic interest in considering lesions studied at necropsy or in surgical material. The descriptions given in this paper give a perspective of the tissue changes seen in the gastro-intestinal tract in a moderately broad selection of material. It is evident that further study of the mucosal changes and of the gastric mucosa in particular is needed.

The second and more immediately practical point of view is that of the value of these criteria in making diagnoses on biopsy material. The differential diagnosis on pathologic grounds between the extension of a malignant process and a radiation reaction may be important. A common example is the problem of an ulcerated rectum in a patient irradiated for carcinoma of the cervix. A pathologic diagnosis of "radiation reaction" rather than "chronic inflammation," if it can be made, may be invaluable in deciding what therapy should be instituted. Therefore a brief review and summary of those changes which are likely to be useful in diagnosis will be presented.

The significant changes may be divided into primary and secondary criteria, that is, those without which the diagnosis cannot be made and those which are merely supportive. It is, of course, obvious that a biopsy specimen submitted for diagnosis may represent only a small part of the lesion. The primary points to be looked for are hyalinization of the connective tissue, abnormal fibroblasts, telangiectasia and hyaline degeneration of vessel walls. These have all been described in detail and illustrated.

Hyalinization of radiation reaction may be simulated by the hyaline fibrous tissue of chronic inflammation, but with experience the peculiar swollen, glassy, afibrillar matrix of radiation reactions can be fairly well distinguished. Rapidly proliferating *fibroblasts* in organizing and healing processes and in active granulation tissue somewhat resemble those seen in radiation lesions. However, in radiation reactions they are less abundant, show little mitotic activity and tend to be stellate and large rather than of the narrow spindle-shaped variety. Abnormal nuclear

forms, too, are rarely seen in other than radiation reactions. *Telangiectasia* involving the veins and lymphatics to a marked degree is very characteristic. It may even be seen in the gross specimen or in the lesion itself prior to removal. The *hyaline degeneration of vessel walls* requires no further comment, although the frequent presence of ordinary arteriosclerosis in the tissues of individuals from the higher age groups may be confusing.

The *secondary diagnostic features* include the endothelial abnormalities, phlebosclerosis, the changes in the muscle fibers and the epithelial alterations. Atypical endothelial cells are seen far less frequently than the almost invariably present altered fibroblasts, but when they are seen, they lend support to the diagnosis. Phlebosclerosis is an important part of the vascular change, but can seldom be seen clearly in a small specimen. It is also seen, of course, in many other types of inflammatory reaction as a residue of phlebitis and consequently is not a specific finding. Stains for elastica may bring out details that would otherwise be missed and many obliterated and partially obliterated veins were overlooked in our preparations until this method was used. The degeneration of the muscle coat may be evident in sections from a complete specimen but is seldom of value in a biopsy fragment.

Finally, the epithelial changes described are not necessarily characteristic of radiation reaction. Atypical glands, hyperplasia, regenerating epithelium and even the mucous change may be seen in any process that has damaged the mucous membrane. However, swollen nuclei with prominent "owl's eye" nucleoli, and marked mucous change are far more frequent in radiation reactions.

NOTE: We are indebted for clinical data to the Surgical Staff of the Palmer Memorial Hospital, particularly Dr. George A. Leland, Jr.; and to the Staff of the Lahey Clinic, as well as to Dr. Joseph H. Marks, radiologist to the New England Deaconess Hospital, and to Dr. Max Ritvo and Dr. F. W. O'Brien.

REFERENCES

1. Friedman, N. B. The stomach and intestine. In: Warren, Shields. The effect of radiation on normal tissues. *Arch. Path.* (In press.)
2. Friedman, N. B., and Warren, Shields. Evolution of experimental radiation ulcers of the intestine. *Arch. Path.*, 1942, **33**, 326–333.

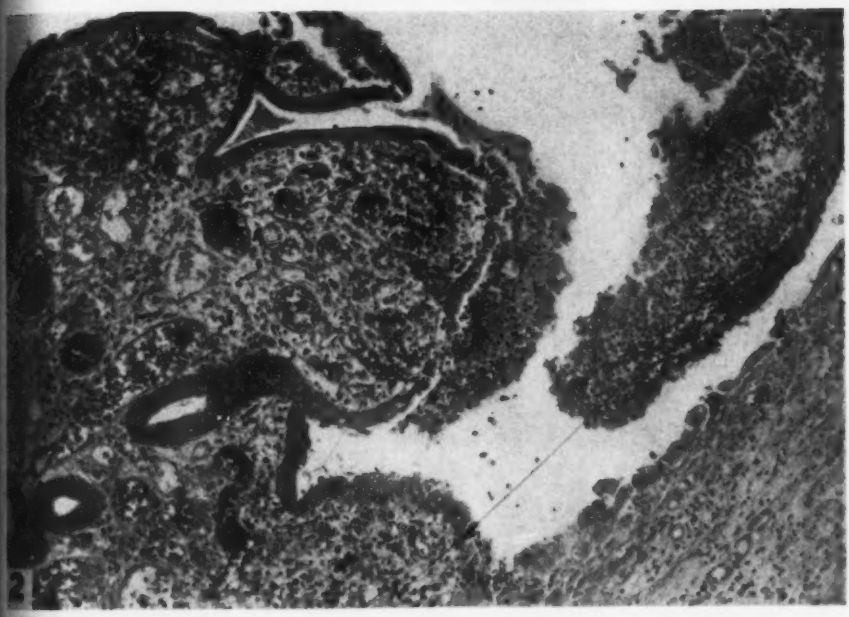
DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Case no. 8. Multiple radiation ulcers and strictures of small intestine.

FIG. 2. Case no. 9. Regenerating epithelium at edge of radiation ulcer of small intestine. × 125.

FIG. 3. Case no. 1. Hyperplastic glands and mucous change in radiation ulcer of stomach. × 125.



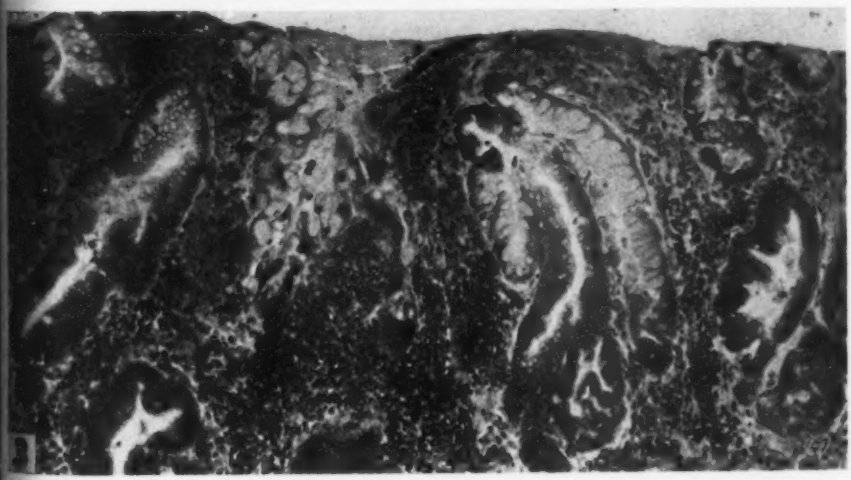


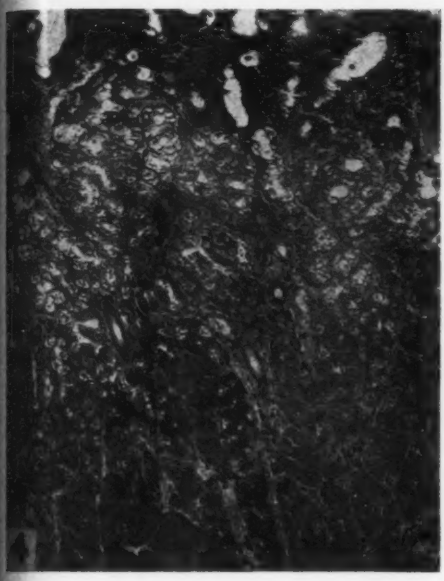
PLATE 83

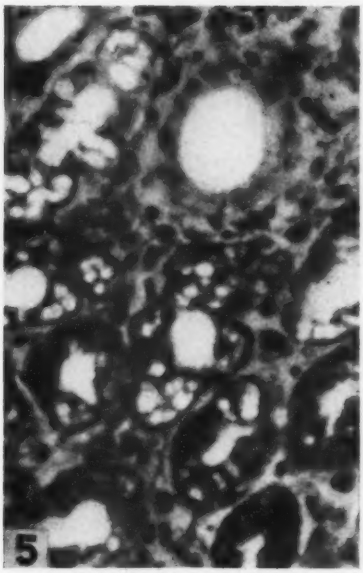
FIG. 4. Case no. 1. Vacuolation of parietal cells in radiation reaction of gastric mucosa. × 125.

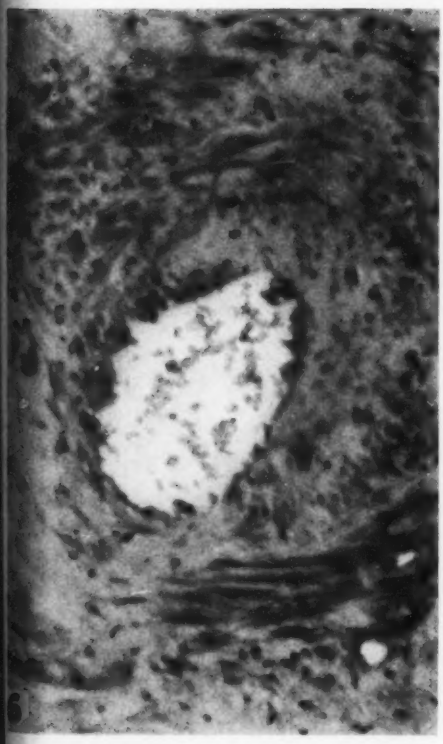
FIG. 5. Case no. 1. Same as Figure 4. × 500.

FIG. 6. Case no. 23. Hyalinized artery in radiation ulcer of rectum. × 200.

FIG. 7. Case no. 8. Phlebosclerosis in radiation reaction of small intestine. Elastica stain. An artery without significant change is seen. × 100.







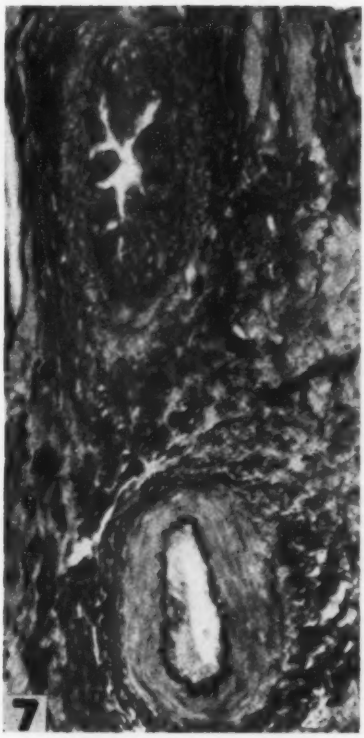
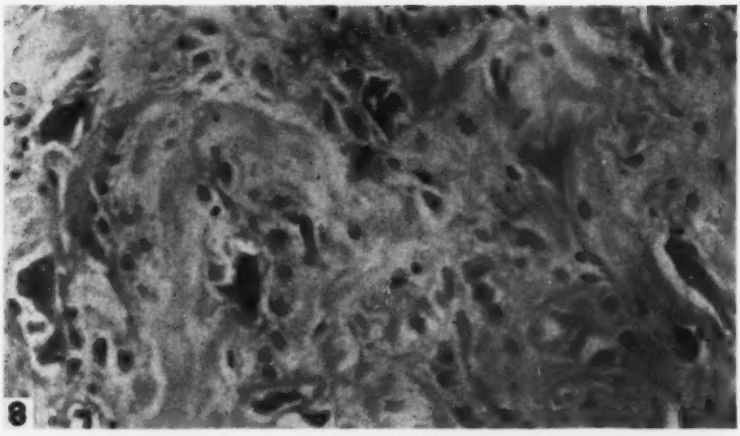


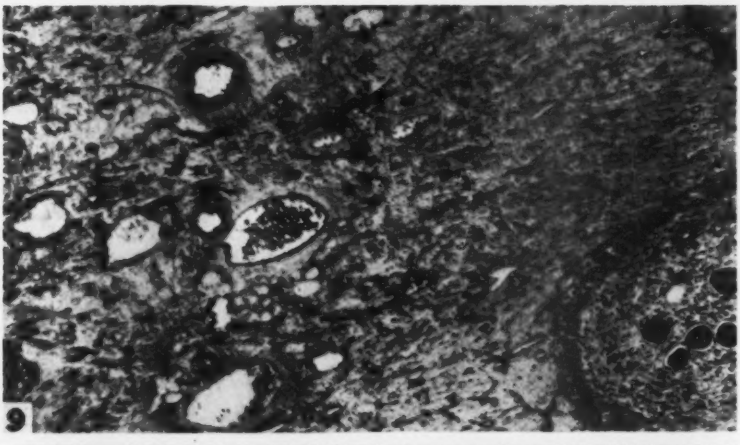
Plate 84

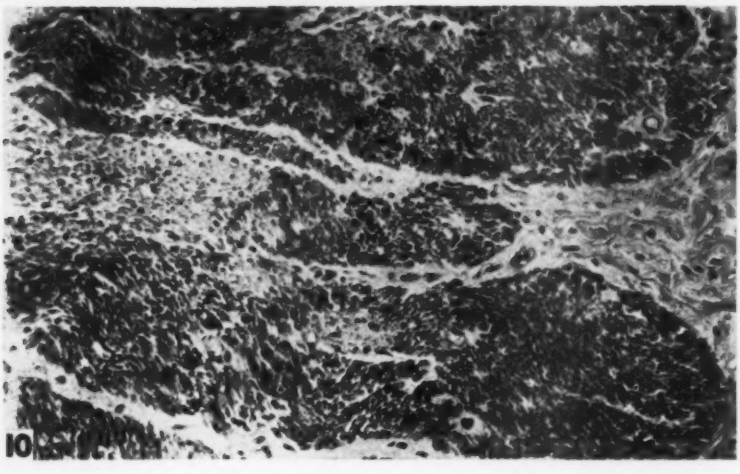
Fig. 8. Case no. 29. Abnormal fibroblasts and hyalinized collagen in radiation ulcer of rectum. × 265.

Fig. 9. Case no. 9. Edema and telangiectasia of submucosa in radiation reaction of small intestine. × 55.

Fig. 10. Case no. 13. Radiation reaction in muscularis of small intestine. × 170.







THE LESIONS PRODUCED IN THE GASTRO-INTESTINAL TRACT BY IRRADIATION *

General Review with an Illustrative Case Report

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The pathologic anatomy of the injury to the gastro-intestinal tract caused by irradiation has been described by a number of investigators. It is the object of this paper to summarize the human cases and the experimental studies from the literature and to add a case of roentgen-ray intoxication of unusual interest. The data included cover only those instances where roentgen rays alone, or in combination with some radioactive substance, have been employed in the treatment of various lesions. Total dosages are stated when available, but the reader is referred to the original articles for other technical details.

Human Cases

Rolleston [1] reviewed the harmful effects of irradiation by both x-rays and radium and listed the symptoms noted after deep irradiation. The severe constitutional symptoms are nausea, uncontrollable vomiting (sometimes hematemesis), offensive diarrhea, melena, abdominal pain and distension, fever up to 104° F., restlessness, profound prostration, progressive cardiac failure, small and rapid pulse and dyspnea.

The first cases with clinical evidence of injury to the gastro-intestinal tract by irradiation were reported by Walsh [2] in 1897. Although deaths were recorded [3] following irradiation therapy, it was not until 1917 that a fatal case with autopsy studies was presented by Franz and Orth.[4] A woman, 35 years old, who had inoperable carcinoma of the cervix, received 2800 units of x-rays in three courses over a period of 5 months. Between the second and third series her weight declined from 112 to 95 pounds. Severe, profuse diarrhea developed after the last series, culminating in death at the end of the fifth month. Necropsy revealed tanning of the abdominal skin, carcinomatous infiltration of the uterus and cervical canal, severe enteritis of the small intestine and ulceration of the large intestine. Microscopic examination revealed heavy cellular infiltrations and hemorrhages in the intestinal ulcerations, masses of bacteria and necrotic tissue, general submucosal edema, cells giving a strong fat reaction with Sudan stain, hyalinization of the mucous membrane, edema and hemorrhage in the muscularis and thickening of the walls and stenosis of the veins.

Von Franqué [5] treated a woman having endometrial hyperplasia with three courses of x-rays. Two weeks after the last series, radiation reaction of the abdominal skin, vomiting, tympanites, profuse, watery diarrhea and severe colic began and subsided slowly. Heck,[6] 16 months later, found the patient to have an intestinal-cutaneous fistula. At operation, friable muscle, thickened peritoneum and deep fascia, extension of the fistula into the cecum and scarring of the ileocecal valve were disclosed. The distal 20 cm. of ileum was resected and the stump of ileum was anastomosed to the side of the ascending colon. After operation the edges of the wound became gangrenous and the sutures cut through, allowing the wound

* Received for publication, August 14, 1941.

to gape. Distension, ileus, intestinal obstruction and peritonitis developed and the patient died 11 days after operation. Autopsy revealed a fibrinopurulent peritonitis and a tight ileocecal anastomosis. The ileum resected at operation was rigid, thickened and edematous. Also observed were widening of the lumina of the glands, atrophy of the lymph follicles, covering of the mucosa by a simple layer of epithelium, absence of the crypts of Lieberkühn, ulceration of the mucosa, disintegration of the muscle coats, foci of lymphocytes in the intestinal wall and thickening and fragmentation of the intima and widening of the media of the blood vessels.

In an analysis of 127 cases treated for various conditions by x-rays, radium, or mesothorium, or by combinations of them, Haendly [7] demonstrated that irradiation can lead to the formation of ulcers of the intestinal mucous membrane and to widespread necrosis of the intestinal wall. The following changes were noted at autopsy: small, punctate, macroscopic hemorrhages; large and small, solitary or multiple ulcers with or without secondary infection; necrosis of the whole intestinal wall; perforation; fistulae; abscesses, and peritonitis. The microscopic changes included capillary dilatation, hemorrhages, stasis, thrombosis, from slight injury to complete necrosis of the vessels, destruction and sloughing of the glandular epithelium, hyalinization of connective tissue and destruction of muscle. Nine cases exhibiting intestinal injury were summarized.

Mathias [8] described a man, 64 years old, having a hypernephroma who was treated with deep x-rays in three series 10 weeks before his death. At autopsy reddening and swelling of the intestinal mucous membrane were found in relation to adhesions to the tumor and the abdominal wall. Microscopically, there were observed sloughed necrotic epithelium and infiltration of the submucosa and muscularis by polymorphonuclear neutrophils.

Fischer [9] added three instances of necrosis of the intestine caused by irradiation. The first case was that of a woman, 53 years old, who had carcinoma of the cervix. Over a period of 49 days she received 1050 X units of x-rays, followed 1 month later by 7288 mg. hours of radium administered over a period of 38 days. Five days after cessation of the radium therapy, the diarrhea, which she had had since the end of the x-ray treatment, stopped and vaginal bleeding commenced. Seventeen days later she died. Autopsy disclosed: ulcerated carcinoma of the portio vaginalis and posterior cervical wall; a rectovaginal fistula; at the left edge of the pelvis beneath an x-ray skin burn, adhesion of a loop of small intestine and friable sigmoid colon; an ulcer in the sigmoid colon, and ulceration of the atrophic and scarred mucosa of the loop of the small intestine.

Fischer's [9] second case was that of a man, 66 years old, who had a large, inoperable tumor of the prostate and was given 1280 X units of x-rays and 1550 mg. hours of radium per rectum, with improvement. Eight and 12 months later, 1820 X and 1280 X units of x-rays, respectively, were given. Sixteen weeks after the last series, marked fever, melena and death with the appearance of urinary sepsis occurred. Autopsy disclosed adenocarcinoma of the prostate, bilateral suppurative nephritis, fibrous peritonitis, a rectal ulcer 6 cm. inside the anus, scarring 3.5 cm. proximal to the ulcer and a mesocolic fistula.

Fischer's [9] third case was that of a woman, 36 years of age, with carcinoma of the cervix who was given an unstated amount of x-ray irradiation over a period of 3 days, with improvement. One month later two more doses of x-rays were given to the parametria. Four months later urinary retention and oliguria were succeeded by severe urinary obstruction and death followed in 2 months. Autopsy disclosed residual carcinoma enveloping the pelvic organs and ureters, acute cystitis, marked hydronephrosis, adhesion of several friable loops of ileum to the abdominal wall, destruction of 40 cm. of the ileal mucous membrane to the ileocecal valve and fibrinous deposit on the under surface of the right lobe of the liver.

Mühlmann and Meyer[10] reported the case of a woman, 56 years old, with cervical carcinoma who had 3450 mg. hours of radium bromide and an unstated amount of x-ray irradiation over a 4 months' period. Seven and 10 months later she was given unstated doses of x-rays to the parametria. At the end of treatment she became bedridden and had melena and anemia with a hemoglobin of 24 per cent. Increased melena and abdominal pain preceded death. Findings at autopsy were: marked anemia; splenic atrophy; great distension of the sigmoid colon which was indurated, adherent to the pelvic wall and to the atrophic uterus, and was involved by a ring of necrosis and perforation 28 cm. proximal to the anus, communicating with a fecal abscess beneath the inguinal ligament, and phlegmon of the wall of the cecum. Microscopically the necrotic parts of the gut were limited by striped tissue overlaid on its inner surface by vessels with marked hyaline thickening or obliteration.

A woman, 42 years old, with hypermenorrhea and marked anemia was studied by Schwarz.[11] She received doses of 100 per cent H.E.D., 122 min., and 90 per cent H.E.D., 131 min., over the anterior and posterior abdominal areas for a period of 4 days. Supportive measures failed to control the ensuing diarrhea, melena and tenesmus. Four months later an abdominal mass of the left lower quadrant was felt. Operation revealed 12 cm. of the sigmoid colon to be white, hard and bound to the thickened left adnexa. A portion of the colon measuring 15 cm. was resected and an end-to-end anastomosis was performed. The resected piece of colon contained several ulcers extending to the serosa and rimmed by necrosis. Necrosis of the mucous membrane at the site of the ulcers breaking through into the submucosa and inner muscle coat, infiltration of inflammatory cells and fibrosis of the submucosa were present on microscopic examination.

A woman, 63 years of age, reported by Fried,[12] had total excision of the uterus for carcinoma followed by deep x-ray irradiation to a sacral and two anterior fields, 110 per cent H.E.D., 50 min., and 100 per cent H.E.D., 118 min. Four weeks later she had the typical appearance of peritonitis, diarrhea, tenesmus and tanning of the abdominal skin. She lost strength rapidly and died. Abdominal autopsy disclosed the following: pneumoperitoneum; intestinal distension; fibrous peritonitis; purulent peritonitis with fecal contamination; rupture of the sacral sigmoid colon; an indurated, stenosed rectum, and mucosal ulceration of the ileocecal valve. Histologically the rectal wall showed marked inflammation of the serosa, edema, foci of chronic inflammatory cells and swelling of the capillary endothelium.

Sanders[13] presented the case of a woman, 39 years old, with general weakness and profuse bleeding who received an unspecified amount of deep x-ray irradiation. Eight days later pigmentation of the skin, diarrhea and chronic ileus were evident. Six months later an operation disclosed a pale, contracted, friable loop of lower ileum adherent to the sigmoid colon and to the linea innominata of the pelvis. An end-to-side anastomosis was done. The resected ileal loop was 45 cm. long, shrunken to half its original volume, firm, thickened and stenosed in two places. One stenotic area was friable and studded with numerous hemorrhages; the other, 10 cm. long, was adherent to the cecum, bean-shaped, taut and dark red. Histologic examination revealed endarteritis and endophlebitis obliterans, nuclear destruction in the glandular epithelium, chronic inflammation and fibrosis in the submucosa and proliferation of vessels in the submucosa and muscularis mucosae.

Ball[14] presented the case of a woman, 41 years of age, with cervical carcinoma who made a good recovery following panhysterectomy. This was followed by a total of 150 e.s.u. of x-rays. The patient developed diarrhea, tenesmus, emaciation, tanning of the abdominal skin, melena and coma and died 38 days later. At autopsy there were observed: a dark chocolate abdominal musculature; adhesions at the site of the operative scar; no evidence of metastases; narrowing and thickening

of 3 feet of jejunum; two partially gangrenous jejunal loops separated by less damaged gut, and hemorrhage into the sacrum. Microscopically the jejunum, ileum and colon showed disintegration and desquamation of the epithelium, hemorrhage into the lumen, atrophy of the lymphatic tissue, fibrosis and an attempt at repair in the completely denuded areas.

Elliott and Jenkinson [15] described a man, 48 years old, who had Hodgkin's disease with general lymphadenopathy, palpable enlargement of the mesenteric lymph nodes, splenomegaly and an epigastric mass. Over a period of 8 months he received 2180 r. in nine series, one to the anterior and one to the posterior abdomen, with 320 r. the maximum dose through any portal. After 2 months, an abdominal mass of the right lower quadrant was discovered. His temperature rose to 104.6° F.; his abdomen became swollen and rigid. A laparotomy showed a fibrinous peritonitis and a swollen appendix which was removed. He died 5 days later. Autopsy revealed the recent laparotomy wound, absence of the appendix, general fibrinous peritonitis and an encapsulated, hemorrhagic perforation on the posterior wall of the stomach. The lymph nodes were enlarged. The stomach contained a dark red blood clot. The posterior surface of the cardia was involved by a large ulcer ringed with satellite ulcers. Two smaller ulcers were on the greater curvature. The ileum showed gray-green ulcerations partially encircling the lumen and distributed from 90 to 200 cm. proximal to the ileocecal valve. The gastric ulcer had a scar and granulation tissue base infiltrated by lymphocytes, monocytes and a few polymorphonuclear neutrophils. The ileal ulcers were edematous and infiltrated by lymphocytes and polymorphonuclear neutrophils. The lymph nodes showed tissue destruction with a slight resemblance to Hodgkin's disease.

Collins and Jones [16] analyzed a series of 422 cases of cervical cancer treated by irradiation therapy. Of these, 6 cases presented the late (4 months to 3 years) complication of benign stricture of the intestine. Five were in the sigmoid colon, 1 in the small intestine. The lesions consisted of an annular fibrous thickening in a localized segment of the intestine associated with varying degrees of constriction of the lumen.

Newell and Crossen [17] listed the complications in the intensive treatment of advanced carcinoma of the cervix by combined roentgen-ray and radium therapy. Five cases of rectal stricture were found.

Todd [18] outlined the case of a woman with cervical carcinoma who received 4000 r. over a period of 4 weeks. During the last week of therapy she developed severe symptoms of enteritis and proctitis and died within 3 weeks. At autopsy patchy ulceration of the small intestine and rectum and histologic necrosis of the mucosa were found. The rectal lesion appeared to be part of a widespread, acute enteritis.

Corscaden, Kasabach and Lenz [19] collected 15 instances of injury to the intestinal mucosa. Among these was an ulcer of the rectosigmoidal colon occurring 2½ months after the end of treatment. At operation the specimen showed necrosis of the entire wall. Another case, 2 months after the end of treatment, disclosed pneumonia, an annular ulcer in the rectosigmoidal colon with microscopic mucosal necrosis, no carcinoma, cloudiness of the submucosal cells and fibrosis of the internal muscle coat with infiltration of plasma cells and polymorphonuclear neutrophils at autopsy. A case of ulceration of the small intestine with onset 2 months after the end of treatment revealed the following at autopsy: fibrinopurulent peritonitis; fibrous adhesions; friable intestinal walls; a thick, hemorrhagic, ileal mucous membrane with microscopic evidence of destruction of all but the crypt epithelium; submucosal edema and inflammation; perimetritis; cervical necrosis, and residual carcinoma.

Ferguson [20] described the lesions in one case of carcinoma of the prostate, one

of carcinoma of the bladder and six of cervical carcinoma. He concluded that irradiation therapy for malignancy of the pelvic organs may produce marked secondary effects on the rectum.

Experimental Studies

Krause and Ziegler [21] exposed mice to x-rays and found in the animals dying spontaneously marked changes in portions of the intestine, including severe catarrh, epithelial desquamation, abundant bacteria in the lumen and crypts and atrophy of lymphatic tissue.

Regaud, Nogier and Lacassagne [22] directed filtered x-rays to the abdomens of six bitches. Desquamation of the epithelium of the small intestine, atrophy of the stroma of the villi, disappearance of the villi and of the glands of Lieberkühn, atrophy of lymphatic tissue, perforation of the severely affected intestinal loops and greater changes in loops more directly in the focus of rays were observed.

In his work on the effect of roentgen and radium rays in guinea pigs and mice, Fromme [23] demonstrated the most severe alterations in the colon and small intestine.

Denis, Martin and Aldrich [24] irradiated rabbits with massive doses of filtered x-rays. A very severe systemic reaction and death were produced only when some portion of the intestinal tract lay within the area of exposure.

Szegö and Rother [25] demonstrated in dogs that human therapeutic doses did not affect the gastric secretion. Massive doses of x-rays to the stomachs of dogs resulted in progressive cachexia, atrophy of the gastric mucosa and burns of the intestinal mucosa.

In guinea pigs, Keller [26] showed that a smaller amount of x-ray irradiation was required to affect the bone marrow and blood than to injure the intestine.

In a series of papers, Warren and Whipple [27] confirmed and extended the work begun by Hall and Whipple.[28] They demonstrated that a massive dose of x-rays over the thorax (up to 512 ma. min.) produced no evidence of clinical disturbance. This was in contrast to the fatal intoxication due to the injury of the small intestine caused by massive doses (350 ma. min.) of x-rays over the abdomen. During the third 24-hour period after exposure, retching, vomiting, bloody and mucous diarrhea, and tenesmus were observed. The small intestine was red and inflamed. The epithelium had nearly vanished, leaving a collapsed framework of edematous mucosa infiltrated by wandering cells. In the fourth 24-hour period, the peak of intoxication and death preceded by coma took place. Histologically, evidence of mitoses and efforts to repair the intestinal epithelium were seen. They considered the epithelium of the intestine as at least as sensitive to x-rays as the lymphocytes of the same area. They showed also that the normal intestinal epithelium undergoes autolysis much less rapidly than irradiated intestinal epithelium, that no bacterial invasion of the body takes place even after the intestinal epithelium has been destroyed by irradiation, that small, repeated doses of irradiation over an interval of 5 or 6 days give a summation effect, that a cone of x-rays may be used to injure part of the gastro-intestinal tract, and that sensitivity to x-rays varies among animals of different species.

Martin and Rogers [29] exposed isolated loops of small intestine of dogs to 75 ma. min. of x-rays. These animals lived from 3 to 5½ months after operation. Before death they evinced anorexia, weight loss, emaciation and cachexia. The x-rayed loops of intestine showed stenosis, thickening and ulceration.

Ivy, McCarthy and Orndoff [30] exposed dogs with Pavlov pouches to one human erythema dose of x-rays of short wave length. All of them had partial or complete anorexia, hyposecretion of gastric juice and anacidity beginning on the third day and continuing for 2 to 3 days. Recovery or death depended on the vitality of

the animal at the time of exposure. The pathologic observations bore out the main findings of Warren and Whipple.[27]

Dawson[31] employed 155 to 205 per cent of a dog erythema dose in irradiating Pavlov pouches and found that the order of injury of the cells of the stomach mucosa of the dog is mucous neck, chief and parietal cells. The superficial one-third of the mucosa was disorganized and the regenerated mucosa had about one-half the thickness of normal. The parietal cells may show no histologic evidence of injury, but be unable to produce acid. The Pavlov pouches showed hypochlorhydria followed by achlorhydria.

Podestà[32] irradiated the abdomens of dogs, with heavy filtration. His observations paralleled those of other workers.[27, 30]

Wolfer[33] produced chronic ulcers in the stomachs of dogs which were similar, grossly and microscopically, to chronic peptic ulcers in man by exposure of the gastric mucosa to roentgen rays. The ulcers did not cause hypersecretion or hyperacidity and when situated near the pylorus, a definite delay in the emptying time of the stomach was noted.

Tsuzuki[34] administered 100 per cent of a human erythema dose to the backs of rabbits. A severe, general systemic reaction and subsequent death were observed. The gastro-intestinal tract showed hyperemia of the mucous membrane, remnants of destroyed nuclei and degenerated lymphocytes, pronounced degeneration of intestinal epithelium at the bottom of the crypts and destruction of lymphatic tissue with phagocytosis.

Lawrence and Tennant[35] found that the effects of neutrons and x-rays on the whole bodies of mice are very comparable. Animals dying more than 4 days after a lethal dose showed bacterial invasion of the viscera. The intestinal mucosa was ulcerated, acutely inflamed, edematous and gave evidence of regeneration of epithelial cells in the glandular crypts.

Report of Case

A Mexican housewife, 42 years of age, was first seen at Colorado General Hospital on August 2, 1940. She presented the following history: intermittent spotting of blood from the vagina for 7 months; pain in the rectum and bladder aggravated by defecation and micturition for 3 months; low back pain, tenesmus and continuous vaginal bleeding for 2 months. On admission her temperature was 99° F. The other vital signs were normal. The patient was crying out with pain and was critically ill. The significant findings were limited to the pelvis. The cervix was nodular, hard and fixed. The parametrial tissues and the rectovaginal and vesicovaginal septa were extensively indurated. The blood hemoglobin was 12.8 gm. (Newcomer); the erythrocytes, 3.3 million per cmm.; total leukocytes, 8700 per cmm.; polymorphonuclear neutrophils, 74 per cent; lymphocytes, 25 per cent; and eosinophils, 1 per cent. The Wassermann reaction was negative. Biopsy of the cervix (Fig. 1) revealed a squamous-celled carcinoma.

The patient was made fairly comfortable by sedation, analgesia and olive-oil enemas. Between August 7th and August 20th, 4200 r. was given to ten anterior and eleven posterior pelvic fields with a 20 by 20 cm. portal, 215 k.v., 15 ma. Thoraeus filter, 50 cm. skin-target distance, 9 minutes per 200 r., and no more than 200 r. over any portal.

On August 16th, nausea, vomiting and diarrhea began. These symptoms continued (although the rectal pain ceased) until August 21st when they became worse, defecation occurring every 5 minutes through the day. Abdominal pain radiating to the back was experienced. On August 20th the patient had a temperature of 99.6° F., a pulse of 128, respirations of 36 and blood pressure of 142/74. Dehydration, thirst and the general critical condition of the patient were obvious. Otherwise

the physical findings were unchanged. The blood hemoglobin was 11.5 gm. (Newcomer); erythrocytes, 3.97 million per cmm.; total leukocytes, 5350 per cmm.; the differential leukocyte count essentially as before. The stool contained blood (++++).

On August 24th the temperature rose to 101° F., the pulse to 200. Sedation, starch enemas and a high caloric-vitamin diet were employed. On August 27th a transfusion of 500 cc. of citrated blood was given. In spite of these measures, intravenous fluids and a Wangensteen apparatus to relieve distension, the nausea, diarrhea and abdominal pain continued. The temperature ranged between 96° and 102.4° F. and the pulse from 115 to 140. Death occurred on September 3rd at 12:45 a.m., 32 days after admission and 14 days after the last exposure to x-rays.

POSTMORTEM EXAMINATION

Autopsy, performed 8 hours after death, revealed absence of pubic hair, reddening of the labia majora and a sacral decubital ulcer, 8 cm. in diameter.

The body cavities, thymus, thyroid and heart were not grossly remarkable. The intima of the aorta was studded with white and yellow plaques measuring 1 to 5 mm. The posterior portion of the left lung was firm and dark red. The right lung was not unusual. The spleen weighed 90 gm., had a smooth, purple-gray surface and a dark red, firm cut surface with obscure markings.

The esophagus and stomach had intact mucosae and thin walls. They contained a watery, opaque, yellow fluid seen also throughout the gastro-intestinal tract. The coils of small intestine were greatly dilated. The duodenum had a yellow, intact mucosa. The mucosa of the jejunum and of the upper part of the ileum was mottled dark red and yellow. The wall was mottled dark red. The appendix was about 7 cm. long and patent throughout. The thickened sigmoid colon and rectum had yellow mucosae studded with fiery red patches. A 3 mm. polyp projected above the mucosa of the cecum.

The pancreas, adrenals, kidneys and liver were not grossly remarkable. The gallbladder contained two yellow, nodular stones, 1.5 cm. in diameter, had a thin wall, a smooth serosa and a mossy mucosa. The urinary bladder had a slightly reddened mucosa.

The uterus was tethered to the left broad ligament by a firm, nodular band extending to the pelvic wall. The uterus was 9 cm. long. The serosa was smooth. The myometrium, 2 cm. thick, contained a white nodule 1 cm. in diameter in the right cornu. The endometrium was 1 mm. thick, yellow and intact. The cervical canal was stenosed. The external cervical os was enlarged, its surfaces black and its left wall very hard. This induration extended into the left broad ligament. The vagina was slightly wrinkled, gray and mottled with black patches. It contained about 10 cc. of opaque, foul-smelling, brown material. The

fallopian tubes and the ovaries were atrophic. The bone marrow was dark red and soft.

The brain weighed 1345 gm., but showed no gross abnormalities.

No evidence of metastasis was demonstrated.

Microscopic Examination

The essential microscopic changes were confined to the gastro-intestinal tract, the genital tract and the bone marrow. The esophagus was well preserved. The stomach showed moderate autolysis of its mucosa. The epithelial covering of the jejunal mucosa was completely absent from the stubby, atrophied villi. The cells of the crypt epithelium were engorged with mucus and showed many mitotic figures (Fig. 3). The mucosal framework was prominent and hyaline streaks coursed through it irregularly. The lymphocyte content of the mucosa was reduced. The lymphatic channels had swollen, hyaline walls. The capillaries were engorged with blood. The colonic mucosa was hyperemic and here, also, the lymphatic channels had swollen, hyaline walls. Small ulcerations (Fig. 4) of the mucosa had necrotic bases infiltrated by a few lymphocytes and macrophages. The floor of the ulcerations was covered with a formless acidophilic material and polymorphonuclear neutrophils. The mucosa of the colon generally was denuded of epithelium and consisted of a thick, mottled, hyaline band infiltrated by small numbers of lymphocytes and macrophages. Occasional giant macrophages were seen. The cells lining the crypts were engorged with mucus and showed many mitotic figures. The submucosa was altered by hyaline deposit and fibrosis. Some of its areas were markedly edematous and were heavily infiltrated by macrophages, some multinucleate. The polyp from the cecum was benign and the overlying mucosa had suffered autolysis.

The hyperemic mucosa of the endocervix was thrown into many papillary excrescences and was heavily infiltrated by chronic inflammatory cells. Dilated, cystic glands were numerous. The mucosa contained blood extravasations penetrated by fibroblasts and areas of fibrosis. Some stretches of the mucosa were covered by a thin, stratified, squamous epithelium. In the wall of the cervix were areas of hyaline necrosis containing bizarre, giant, anaplastic epithelial cells with uneven cytoplasmic borders and huge irregular masses of chromatin indicative of injured nuclei (Fig. 2).

The vaginal epithelium was denuded, laying bare a very vascular granulation tissue crowded with lymphocytes and plasma cells and overlaid by necrotic, hyaline débris and polymorphonuclear neutrophils. In the vaginal wall were large areas of hyaline necrosis including small clumps of tumor-cell ghosts.

The bone marrow of the rib had a normal cellularity. A smear showed an almost complete disappearance of segmented, stab and metamyelocyte neutrophil forms with a marked increase in myelocyte and promyelocyte neutrophils and stem cells. Eosinophils and lymphocytes were present in about normal numbers. The erythrocyte line was relatively decreased.

The main anatomical diagnoses were: roentgen-ray intoxication, carcinoma of the cervix (residual, post-irradiation), chronic ulcerative cervicitis with squamous epithelialization, chronic suppurative vaginitis, maturation arrest of neutrophilic granulocytes in the bone marrow.

Comment

The parallel between the clinical symptoms and the pathologic findings in irradiation damage of the gastro-intestinal tract in humans and in animals is readily apparent. The main gap, as in other human diseases, is the difficulty of obtaining tissues, either postmortem or by surgical excision, demonstrating the pathogenesis of the early stages.

Since gastro-intestinal upset commonly follows irradiation of lesions in or adjacent to the abdominal cavity, it is well to remember that an occasional fatality will result, even with doses usually found to be not excessive, such as the 4200 r. employed in my case. The only reported case closely resembling it was that of Todd.[18] This patient also had cervical carcinoma and received 4000 r. Probably more cases of this type than have been recorded have died as a result of irradiation therapy with or without a contribution toward the cause of death by the disease for which the irradiation was used. Probably also the great majority of these cases succumb outside an institution with lessened likelihood of permission for autopsy.

With the use of filtration and several portals of exposure on a deep-seated lesion, skin injury may be relatively slight, but the underlying tissues may be severely irradiated. This is especially disastrous to the intestinal epithelium, which has been considered as sensitive as the lymphocytes in the same area,[27] but less susceptible than the bone marrow and the blood[26] to irradiation. In addition to the immediate destruction of the intestinal epithelium, the more distant effect of irradiation on the blood vessels of the intestine, with thrombosis and infarction of the whole thickness of its wall, must be borne in mind as well as the sequelae of ulceration, perforation, abscess formation, fistulae, peritonitis, fibrosis, stenosis and intestinal obstruction. Similarly, tumor cells may be killed either by their inherent sensitivity to irradiation, or being relatively insensitive, by choking off their blood supply through vessel damage.

In determining the location of x-ray injury to the gastro-intestinal

tract, the sites of predilection are those anatomically or pathologically fixed in the path of the x-ray beams. These include loops of small intestine fixed by adhesions, the distal 50 cm. of the ileum, the cecum, the sigmoid colon and the rectum.

Probably the presence or absence of evidences of terminal bacterial invasion of the blood and lymph streams and the viscera is conditioned by both the size of the animal and by the length of time elapsing after death before postmortem examination is performed.

The phenomenon of roentgen-ray cachexia[29] is well explained by the failure of food absorption from the damaged gastro-intestinal tract. Data not included in the record of our patient indicate that she had a ravenous appetite, but was losing weight to an unknown amount (weight at autopsy, 81 pounds) during the course of treatment.

Conclusion

A case of roentgen-ray intoxication is presented, with the typical clinical and pathologic pictures of other reported human cases, particularly those exhibiting symptoms during a first and only course of irradiation directed to or near the abdominal cavity and dying within a few weeks after treatment. The human cases and the experimental studies from the literature dealing with the pathologic anatomy of irradiation damage to the gastro-intestinal tract have been reviewed.

REFERENCES

1. Rolleston, Humphrey. The harmful effects of irradiation (x-rays and radium). *Quart. J. Med.*, 1930–31, **24**, 101–131.
2. Walsh, David. Deep tissue traumatism from roentgen ray exposure. *Brit. M. J.*, 1897, **2**, 272–273.
3. Edsall, D. L. The attitude of the clinician in regard to exposing patients to the x-ray. *J. A. M. A.*, 1906, **47**, 1425–1429.
4. Franz, K., and Orth, J. Fall einer Röntgenschädigung. *Berl. klin. Wchnschr.*, 1917, **54**, 622–664.
5. Von Franqué, Otto. Schwere Darm- und Hautschädigung bei Röntgentiefentherapie mit Schwerfilter. *Zentralbl. f. Gynäk.*, 1918, **42**, 1–4.
6. Heck, W. Schwere Darm- und Hautschädigung bei Röntgentiefentherapie mit Schwerfilter. *Strahlentherapie*, 1920, **11**, 796–802.
7. Haendly, P. Pathologisch-anatomische Ergebnisse der Strahlenbehandlung. *Strahlentherapie*, 1921, **12**, 1–87.
8. Mathias. Röntgenverbrennungen des Dickdarms. *Centralbl. f. allg. Path. u. path. Anat.*, 1922–23, **33**, 65.
9. Fischer, Bernh. Über Bestrahlungsnekrosen des Darmes. *Strahlentherapie*, 1922, **13**, 333–358.
10. Mühlmann, E., and Meyer, O. Beiträge zur Röntgenschädigung tiefgelegener Gewebe. *Strahlentherapie*, 1923, **15**, 48–64.
11. Schwarz, Egbert. Über Schädigungen bei der Röntgenbehandlung von Myomen und hämorrhagischen Metropathien. *Strahlentherapie*, 1923, **15**, 398–411.

12. Fried, Carl. Ein Todesfall durch Darmruptur nach Röntgen-Tiefenbestrahlung. *Strahlentherapie*, 1923, **14**, 688–706.
13. Sanders, Arthur. Zur Kenntnis der Röntgenschädigungen am Darm. *Strahlentherapie*, 1924, **18**, 457–472.
14. Ball, C. F. Abdominal deep therapy injuries. With report of necropsy findings in a fatal case. *Am. J. Roentgenol.*, 1925, **13**, 220–226.
15. Elliott, A. R., and Jenkinson, E. L. Ulcerations of the stomach and small intestine following roentgen therapy. *Radiology*, 1934, **23**, 149–156.
16. Collins, E. N., and Jones, T. E. Benign stricture of the intestine due to irradiation of carcinoma of the cervix uteri. *Surg., Gynec. & Obst.*, 1934, **59**, 644–649.
17. Newell, Q. U., and Crossen, H. S. Late complications in irradiation treatment of cancer of the cervix. *Surg., Gynec. & Obst.*, 1935, **60**, 763–768.
18. Todd, T. F. Rectal ulceration following irradiation treatment of carcinoma of the cervix uteri. Pseudocarcinoma of the rectum. *Surg., Gynec. & Obst.*, 1938, **67**, 617–631.
19. Corscaden, J. A.; Kasabach, H. H., and Lenz, Maurice. Intestinal injuries after radium and roentgen treatment of carcinoma of the cervix. *Am. J. Roentgenol.*, 1938, **39**, 871–894.
20. Ferguson, L. K. Secondary effects of radiation upon the rectum following the treatment of extrarectal pelvic lesions. *Am. J. Digest. Dis. & Nutrition*, 1937–38, **4**, 712–719.
21. Krause, Paul, and Ziegler, Kurt. Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf tierisches Gewebe. *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1906–07, **10**, 126–182.
22. Regaud, C.; Nogier, T., and Lacassagne, H. Sur les effets redoutables des irradiations étendues de l'abdomen et sur les lésions du tube digestif déterminées par les rayons de Röntgen. *Arch. d'électric. méd.*, 1912, **21**, 321–334.
23. Fromme, Georg. Ueber die allgemeine Wirkung der Röntgen- und Radiumstrahlen. *Ztschr. f. Geburtsh. u. Gynäk.*, 1916–17, **79**, 579–613.
24. Denis, W.; Martin, C. L., and Aldrich, M. A study of the relative toxic effects produced by regional radiation. *Am. J. M. Sc.*, 1920, **160**, 555–567.
25. Szegö, Eugen, and Rother, Julius. Über den Einfluss der Röntgenstrahlen auf die Magensaftsekretion. *Ztschr. f. d. ges. exper. Med.*, 1921, **24**, 270–288.
26. Keller, Ernst. Die Wirkung intensiver Röntgenbestrahlung auf den Darm und ihre Folgeerscheinungen. *Ztschr. f. exper. Path. u. Therap.*, 1921, **22**, 284–309.
27. Warren, S. L., and Whipple, G. H. Roentgen ray intoxication. *J. Exper. Med.*, 1922, **35**, 187–224. *Ibid.*, 1923, **38**, 713–752.
28. Hall, C. C., and Whipple, G. H. Roentgen-ray intoxication: disturbances in metabolism produced by deep massive doses of the hard roentgen rays. *Am. J. M. Sc.*, 1919, **157**, 453–482.
29. Martin, C. L., and Rogers, F. T. Roentgen-ray cachexia. *Am. J. Roentgenol.*, 1924, **11**, 280–286.
30. Ivy, A. C.; McCarthy, J. E., and Orndoff, B. H. Studies on the effect of roentgen rays on glandular activity. IV. Effect of exposure of abdominal and thoracic areas to roentgen rays on gastric secretion; note on roentgen cachexia. *J. A. M. A.*, 1924, **83**, 1977–1984.
31. Dawson, A. B. Histological changes in the gastric mucosa (Pavlov pouch) of the dog following irradiation. *Am. J. Roentgenol.*, 1925, **13**, 320–326.
32. Podestà, Vittorio. Studi sperimentali sulle lesioni intestinali da raggi X nella applicazione della radioterapia profonda intensiva. *Radiol. med.*, 1925, **12**, 201–216.

33. Wolfer, J. A. Chronic experimental ulcer of the stomach. Its clinical significance. *J. A. M. A.*, 1926, **87**, 725–729.
34. Tsuzuki, Masao. Experimental studies on the biological action of hard roentgen rays. *Am. J. Roentgenol.*, 1926, **16**, 134–150.
35. Lawrence, J. H., and Tennant, Robert. The comparative effects of neutrons and x-rays on the whole body. *J. Exper. Med.*, 1937, **66**, 667–688.

DESCRIPTION OF PLATE

PLATE 85

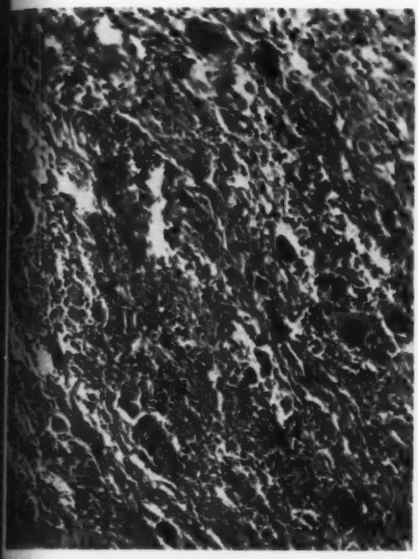
FIG. 1. Squamous-celled carcinoma of cervix, before x-ray treatment. × 130.

FIG. 2. Residual carcinoma of cervix, after x-ray treatment. × 130.

FIG. 3. Detail of jejunal gland with evidence of regeneration of epithelium. × 190.

FIG. 4. Ulceration of colon. × 35.

MERICAN
Mulligan



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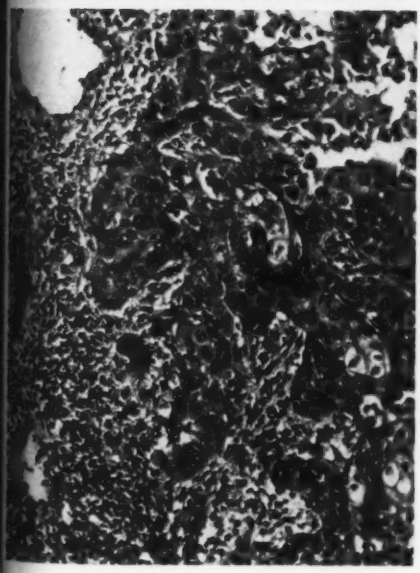

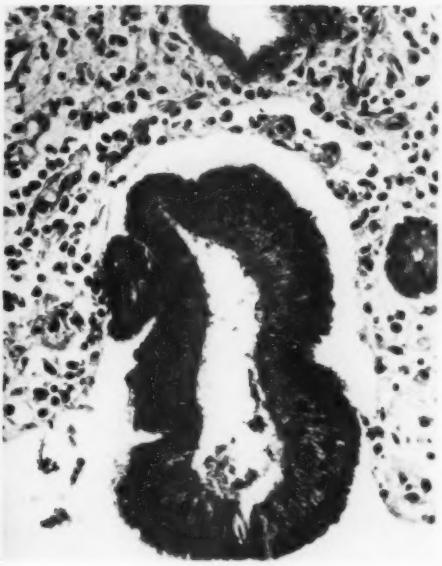

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SYNOVIAL SARCOMESOTHELIOMA (SARCOENDOTHELIOMA) *

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There is a malignant tumor of the synovial membranes which exhibits an apparent pluripotentiality in definitive cellular development, recognized by the presence of both connective tissue and epithelial-like cellular formations. Although Stüer, in 1893, first reported this tumor under the descriptive term of adenosarcoma, it was not until 1910 that Lejars and Rubens-Duval described and interpreted it as a peculiar tumor of the synovial membrane, giving it the name *endothéliome synovial*. The tumor's relatively complex microscopic structure has led a number of subsequent authors to give it various names. Chenot and Tzanck called it an *alveolar carcinoma,* Enderlen a *perithelial sarcoma* or a *sarcocarcinoma*. Smith, after considering the names endothelioma and mesothelioma, chose *synovioma;* Sabrazès and de Grailly (1931) modified it to *synovialoma,* and Wegelin (1928) finally selected the term *synovial sarcoendothelioma*.

Reports of Cases

Case 1

A. B., a female stenotypist, 20 years old, had observed a swelling in the right popliteal space "for some time" (months). Pains, caused by complete extension of the leg and occurring at night, induced her to seek medical attention in the middle of August, 1934. She recalled injuring her right knee by a fall while ice-skating when a child, but this is of doubtful significance. No definite preoperative diagnosis of the tumor was made. X-ray study showed the bone uninvolved. Although attached to the periarticular structures, because of its definite margins the tumor was considered benign and so was locally excised on August 23rd. During the operation the surgeon could find no definite attachment to the knee joint capsule or to the overlying skin and thought that the tumor arose from some deep structure near the popliteal vessels. Healing was prompt.

Pathologic Report on Original Tumor. The tumor, 6 cm. in diameter, had an irregularly nodular surface. Section showed it to be made up of a light gray, granular and fibrous tissue that was firm except for some small areas of softening with hemorrhagic infiltration. A milky juice could be scraped from the cut surface. Histologically the connective tissue stroma was found to be very cellular for the most part. Epithelial-like cells occurred as solid cords, as the lining of large and small spaces and as a regular layer covering connective tissue papillae

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(Fig. 1). These cells had a small nucleus, rich in chromatin, and where the cells were arranged in a layer the cytoplasm was small in amount and definitely eosinophilic. With the mucicarmine stain the contents of the cystic spaces stained as true mucus. Sections from the surface showed a distinct connective tissue capsule, in no place broken through by the tumor.

This tumor was originally diagnosed as an adenomatous neoplasm, possibly taking its origin from a dermal appendage. Subsequent discussion between the clinician and the pathologist indicated that this diagnosis was not considered as completely final; it was ventured that it might be a mixed tumor; in any case it was considered benign.

Clinical Course. The patient remained symptomless until the early part of the following year when occasional tearing pains in the leg were noted. By the beginning of May she noticed an enlargement at the site of the previous operation. Clinical examination, on June 17th, showed a tumor 7 by 4 by 3 cm. in size, in the popliteal space, under the upper half of the old scar. The tumor was hard and attached to the deeper structures; the overlying skin was only slightly fixed, but contained numerous, visible blood vessels. Inguinal lymph nodes were not enlarged. This recurrence was removed on June 23rd. As the tumor had to be dissected from the deep vessels the surgeon did not consider it as a radical removal. At operation the appearance of the neoplastic tissue suggested sarcoma. The wound healed directly, allowing complete freedom of motion of the knee joint.

Pathologic Report on First Recurrence. An oval mass of tissue, measuring 6 by 4 by 3 cm., was covered, for the most part, by loose connective tissue and fat (Fig. 2). The cut surface was partly reddish gray, soft and homogeneous; partly that of a finely villous, gray-white tissue that was subdivided into areas of various sizes by delicate connective tissue septa. Here and there the tissue contained small spaces, up to 2 mm. in diameter, filled with a moderately viscid, colorless fluid. The tumor was infiltrated by fresh hemorrhage in numerous places and its central portion was brownish in color. Histologic examination showed that the neoplastic tissue was formed of spindle and oval cells with oval nuclei. Mitoses were frequent. Small blood vessels were numerous. Special staining methods demonstrated a fibrillary interstitial substance between these cells. This tissue was arranged in papillary formations which were covered by epithelial-like cells, which here and there showed a definite cylindrical form (Fig. 3). Reticulin stains showed no interstitial fibers between these epithelial-like cells. Their boundary against the stroma was for the most part sharp; in some places, however, the cells of the stroma appeared to merge with the epithelial-like elements (Fig. 4). Staining with mucicarmine revealed a true mucous substance in the clefts between the papillary structures and in small glandlike spaces lined by the epithelial-like cells. The histologic diagnosis was synovial sarcoendothelioma.

Subsequent Clinical Course. Within 3 months there was recurrence on each side of the scar, with limitation of extension of the knee joint. Amputation was advised but the patient declined. At this time there was no loss of weight and no detectable lesions except in the region of the right knee. The tumors grew so rapidly that by January 25, 1936, one nodule was the size of an apple, the other walnut-sized. Both were firm and painless. The overlying skin was livid and perforated by pinhole ulcerations that exuded a bloody fluid. The inguinal lymph nodes were moderately enlarged, firm and painless. X-ray study showed pulmonary metastases and atrophy of the distal end of the femur. Intensive x-ray treatment of both the local growth and the lungs was instituted. The popliteal tumors continued to grow, becoming soft and widely ulcerated over their surfaces. In roentgenograms taken on March 30th the pulmonary metastases were no longer visible. The patient finally consented to radical operation. The right leg was amputated just below mid-thigh on April 8, 1936.

Pathologic Report on the Amputated Right Lower Extremity. Two tumor masses were found in the upper popliteal region, one arising from each side of a widened longitudinal scar. The medial mass measured 13 by 11.5 by 7 cm.; the lateral one, not so large, 8.5 by 6 by 4 cm. Their discolored, ulcerated surfaces were covered with pus; the surrounding skin was thin and adherent to the underlying tumors. A deep extension of the tumor was found between the vascular bundle and the femur, almost to the line of amputation. A median longitudinal section through the femur and tibia showed the combined tumor mass filling the entire popliteal space (Fig. 5), extending to but not involving the femur. The bone was unchanged except for an increased porosity (atrophy). The cut surface of the tumor showed it to be fairly sharply demarcated from the surrounding soft parts. It was formed of a gray-white tissue, for the most part fibrous in texture, divided into large areas by wide connective tissue septa. With the naked eye small cysts could be seen, not exceeding the size of a pepper seed and filled with a colorless fluid, located in the fibrous neoplastic tissue. Superficially there were larger smooth-walled cysts, up to 1 cm. in size, some filled with a brown-red fluid, others by dark red blood clots. Especially in the cranial pole of the tumor and over its ulcerated surface were found irregularly marginated, yellow foci of necrosis and an old hemorrhagic infiltration. Histologically the tumor had the same appearance as that noted in the previous report of June 24, 1935.

Terminal Clinical Course. The amputation stump healed with fair rapidity, and recurrence in the stump could never be determined by clinical examination. Intensive x-ray therapy to the stump and chest was continued at intervals. Large metastases were again seen in the pulmonary roentgenograms on May 19th. Pains in the chest, a continuous irritative cough, dyspnea and finally hemoptysis developed. The patient died on March 16, 1937, after an illness lasting 2 years and 7 months.

Pertinent Postmortem Findings. The skin of the thoracic region was pigmented brown (x-ray therapy). The transverse scar on the distal

end of the amputation stump was completely healed. The inguinal lymph nodes were not enlarged. No neoplastic tissue was present in the dissected stump or regional inguinal nodes.

The left lung was partly adherent by fibrous pleural adhesions over the lower lobe and the inferior portions of the upper lobe. In the middle of the left lower lobe there was an oval cavity, 13 by 4 by 4 cm. in size, filled with necrotic neoplastic tissue and ichorous fluid. A similar cavity was located at the base of the left lung on the diaphragmatic surface. Near the apex of the left lower lobe there was a sharply circumscribed nodule, 6.5 by 3.5 by 3 cm. in size, of a gray-white, unusually soft, homogeneous tissue, with areas of necrosis and fresh hemorrhage. Several similar tumor metastases were scattered throughout the remainder of this lung. In the phrenico-costal angle of the right lower lobe there was a partly degenerated tumor metastasis, about 5 cm. in diameter, similar in appearance to that in the left lower lobe. Also throughout the right upper lobes there were a number of smaller tumor nodules. In the middle and upper lobes there were numerous foci of lobular pneumonia. The mediastinal lymph nodes under the bifurcation and along the trachea were succulent and anthracotic, but contained no neoplastic tissue.

The remainder of the postmortem examination, which was complete and included the head, revealed no other tumor deposits. Death was caused by lobular pneumonia complicating the degenerating pulmonary metastases.

Microscopic Examination of the Pulmonary Tumor Deposits. Most of the sections, taken from several nodules, had an adenomatous appearance (Figs. 6 and 7). Round, oval or elongated spaces were lined by an epithelial-like layer of cells. This layer was in some places formed by a single row of cells; in others the lining stratum was multilayered. These epithelial-like cells were columnar or polygonal in form, with uniformly medium-sized, round or oval nuclei that seldom showed mitotic figures or polymorphology and contained only a moderate amount of fine, scattered chromatin. The cytoplasm was clear, eosinophilic and rather sparse. The epithelial-like structures were supported by a sarcomatous tissue that was very variable in amount and irregularly distributed in the various nodules. In some few areas it occurred in large sheets (Fig. 8). It was richly cellular with uniform, rather vesicular, oval nuclei. Few intercellular fibrils were seen in most areas other than the solid sheets, although they could be demonstrated readily by special stains. Mitotic figures were infrequent. The epithelial-like layer was usually definitely demarcated from the subjacent sarcomatous stroma; indeed in some places the supporting cells seemed to be

tangentially arranged, but a definite basement membrane was lacking. Moreover, in a few situations the sarcomatous cells merged into the epithelial-like, lining cell-layer with uninterrupted transition. The contents of the glandlike spaces and clefts stained red with mucicarmine. The various nodes had the sharply delimited margin of growth of metastases. Sections from the wall of a large cavity in the left lower lobe showed widespread necrosis of neoplastic and pulmonary tissue with edema and inflammatory infiltration of the marginal tissue of the lung.

The initial impression on cursory examination of these sections was that of adenocarcinomatous metastases. When attention was given to the specialized sarcomatous stroma, the association of it with the epithelial-like structures and the finer cytologic details of both components, the diagnosis of pulmonary metastases of a synovial sarcoendothelioma was readily made, especially in view of the previous findings in the case.

The salient features of this case are the characteristic clinical course of this tumor in the region of the knee joint, with repeated recurrences after operation and ultimate spread to the lungs, and the noteworthy histologic appearance of the pulmonary metastases. The complex structure of synovial sarcoendothelioma found in the original tumor was repeated in both recurrent masses and in the lung deposits.

The rarity of these tumors prompts me to report another case, of which the termination is as yet unknown.

Case 2

(Clinical report through the courtesy of Dr. E. Stumme, Znaim, Austria.) Recent growth in a preëxisting tumor in the popliteal fossa of M. E., a housewife, 56 years old, led to an original diagnosis of malignant degeneration of a "ganglion." At operation on October 22, 1938, the tumor was found to have an ill-defined, infiltrating margin, with attachment to the sheath of the popliteal vessels demanding amputation. After the operation the surgeon's opinion was that he was dealing with a malignant hemangioma.

Pathologic Report. The tumor, isolated from the popliteal fossa of the amputated leg, was irregular in shape, and measured 10.5 by 5.5 by 4 cm. In its interior was found a whitish tissue with areas of hemorrhagic infiltration. The margin was poorly defined, for the tumor invaded the neighboring tissue including the sheath of the popliteal artery. Histologic study of sections from this tumor showed the combination picture characteristic of sarcoendothelioma (Fig. 9). Irregularly shaped clefts and spaces were lined by a tissue that was most epithelial-like. Usually this layer was many cells in thickness; only occasionally was it a unicellular stratum. Mitoses were frequent. These

formations were in turn embedded in a very cellular sarcomatous stroma in which the fusiform elements had oval nuclei. Intercellular fibers were present but not abundant. Frequently the two varieties of cellular formations merged with one another. In one section the tissue supporting the layer lining the spaces was hyalinized. Many areas were necrotic. With the mucicarmine stain the clefts and glandlike spaces were seen to contain mucus.

Literature

A review of the literature of synovial tumors reveals 43 cases which can be classified as synovial sarcoendothelioma. The criterion for this selection was a bimorphic microscopic appearance, each of these tumors exhibiting both a connective tissue element and a tissue that may be described as epithelial-like, usually found lining small glandlike or cystic spaces or clefts, covering papillary projections or arranged in cords or islands. As a guide in the adjudication of synovial tumors, the descriptions of joint tumors as given by Chiari was followed. The collected cases, with the 2 reported in this paper, are listed in Table I.

In addition to a multiplicity of names, the literature pertaining to sarcoendothelioma is further confused by the previous acceptance of cases which on strict micro-morphologic grounds can hardly be admitted to this group. A number of reviews of this subject have started with a discussion of the older reports of sarcoma of the joints, and continuing into the cases of sarcoendothelioma have drawn no definite line of separation. Frequently the name given a reported tumor is so nonspecific, such as "sarcoma," that the entire case must be reviewed to determine its exact nature.

The connotation of the term sarcoendothelioma has varied with different authors. An illustration to point is the paper by Coley and Pierson in which 15 cases of "synovioma" are reported. According to Smith, who originated the term, synovioma is this peculiar bimorphic tumor with which we are dealing. One might then presume that all of Coley and Pierson's cases were of this variety, but Stewart, who was responsible for at least the majority of the microscopic diagnoses on these tumors, considered the term synovioma to include sarcoendothelioma, giant cell tumor and xanthoma, and sarcoma, when arising from synovial membrane. Giraud, Salmon and Paillas gave synovialoma a similar scope. Geschickter and Lewis classed synovioma with giant cell tumor and xanthoma, and gave it no discussion as an entity.

To save future repetitious bibliographic investigation, the cases not considered as sarcoendothelioma for one of the above reasons will be found in the appended supplementary bibliography.

In 1902, Spencer referred to a sarcoma of the synovial membrane as an endothelioma. During the last decade this name has been applied to a number of cases, and they comprise a group that gives the greatest difficulty in classification. In this category we would place the cases reported by Harbitz, case no. 1, 1927; Wagner, two cases, 1930; Hodgson and Bishop, 1935; von Rosen, 1937 (Jönsson, case no. 82); Coley and Pierson, cases nos. 5 and 10, 1937; possibly that reported by Janik, case no. 5, 1927; and Bogetti's angioendothelioma, 1937. These tumors exhibit elements that have grown as sheets of cells with a fair amount of cytoplasm, little

TABLE I

Reported Cases of Sarcoendothelioma

No.	Author	Year	Age	Sex	Location	Origin		
						Joint	Tendon sheath	Bursa
1	Stüer	1893	21	M	R. elbow	+		
2	Burckhardt	1909	46	M	Knee	+		
3	Lejars and Rubens-Duval	1910	22	M	L. knee	+		
4	Chenot and Tzanck	1912	38	F	L. foot	+		
5	Enderlen	1920	40	F		+		
6	Smith, case no. 1	1927			Thigh		?	?
7	Smith, case no. 2	1927	24	F	Thigh		?	?
8	Smith, case no. 3	1927	35	M	Knee	+		
9	Wegelin	1928	28	M	L. knee	+		
10	Schwamm	1930	13	F	Shoulder			+
11	Tavernier	1930	23	F	Knee	+		
12	Diez	1931	33		R. knee	?	?	
13	Prym	1931	66	F	R. knee	+		
14	Sabrazès, Loubat, de Grailly and Magendie, case no. 1	1932	18	F	L. knee	+		
15	Sabrazès, *et al.* (Baillis and Bonnard), case no. 2	1932	50	M	L. elbow	?	?	
16	Bonne and Collet	1935	25	F	L. knee			+
17	Fievez	1935		F	L. ankle	+		
18	Zwahlen, case no. 1	1935	22	F	L. wrist		+	
19	Zwahlen, case no. 2	1935	16	M	Ankle		?	?
20	Black	1936	36	M	R. hand		?	?
21	Brunner	1936	54	M	R. up. arm			+
22	Knox, case no. 1	1936	22	F	R. elbow	+		
23	Knox, case no. 2	1936	33	M	R. knee	?	?	?
24	Fehr	1937	18	M	R. thumb			+
25	von Verebély	1938	51	M	Knee	+		
26	Jönsson, case no. 1 (#71)	1938	50	F	L. wrist		+	
27	Jönsson, case no. 2 (#72)	1938	20	M	Wrist		+	
28	Jönsson, case no. 3 (#73)	1938	23	F	L. axilla			+
29	Jönsson, case no. 4 (#74)	1938	54	M	L. shoulder			+
30	Jönsson, case no. 5 (#75)	1938	40	M	L. up. arm			+
31	Jönsson, case no. 6 (#76)	1938	53	M	Hand		+	
32	Jönsson, case no. 7 (#77)	1938	70	F	L. thigh			+
33	Jönsson, case no. 8 (#78)	1938	40	F				+
34	Jönsson, case no. 9 (#79)	1938	14	M	L. hand		+	
35	Jönsson, case no. 10 (#80)	1938	29	F	R. 4th toe		+	
36	Jönsson, case no. 11 (#81)	1938	37	F	R. temporal			+
37	Jönsson, case no. 12 (#83)	1938	37	F	R. shoulder			+
38	Jönsson, case no. 13 (#84)	1938	69	F	R. hand		+	
39	Berger, case no. 1	1938	30	M	L. thigh			+
40	Berger, case no. 2	1938	38	M	L. thigh			+
41	Berger, case no. 3	1938	26	M	R. axilla			+
42	Franseen, Simmons and Mallory	1939	71	M	R. knee		?	
43	Albot, Thiebaut, Banzet and Hervy	1939	52	F	R. elbow	+		
44	Fisher, case no. 1	1942	20	F	R. knee		?	?
45	Fisher, case no. 2	1942	56	F	Knee		?	?

TABLE II

Features of the Reported Cases of Sarcoendothelioma

Case	History of trauma	Initial symptom: Pain	Initial symptom: Swelling	Initial symptom: Limitation of motion	Duration of symptoms before obtaining treatment (yr. mo.)	Treatment: Biopsy	Treatment: Local excision	Treatment: Radical excision	Treatment: Amputation	Treatment: X-ray	Interval from first treatment to termination (yr. mo.)	Local recurrence	Metastases: Lymph node	Metastases: Lung	Bimorphic microscopic structure: Recurrence	Bimorphic microscopic structure: Lymph node	Bimorphic microscopic structure: Lung	Reported termination
Stüer			+		3		+						+					
Burckhardt	+		+				++											
Lejars and Rubens-Duval		+			6		+	+	+			++			+			dead
Chenot and Tzanck	−		+		o—8		+											
Enderlen							+											
Smith, case no. 1							+				o—6			C*				dead
Smith, case no. 2	−	+			o—8		+			+	2—5	+	+	C		−		dead
Smith, case no. 3			+		o—5		+		+		2—1	+		C	−			dead
Wegelin			+		o—3		+	+			4—11	+		+			−	dead
Schwamm		+					+							C				dead
Tavernier				+			+	+				+						
Diez	+	+			5		+											
Prym		+			7		+		+	+	2—5	+	+	−				dead
Sabrazès *et al.*, case no. 1	+	+			5		++		+	+	2	+	+	C				dead
Sabrazès *et al.*, case no. 2			+		4		+											dead
Bonne and Collet							+				1—1	−	−	−				living
Fievez	+		+						+									
Zwahlen, case no. 1		+			6	+	+		+	+	3—6	+		C	−			dead
Zwahlen, case no. 2			+		1		+	+	+		3—5	++		+				dead
Black	+		+				+											living
Brunner			+		7		+				3		+	C				dead
Knox, case no. 1		+			1—6	+	+	+	+		8—6	+	?	C	+	+		dead
Knox, case no. 2			+		o—6	+			+		1—7			C				dead
Fehr	−		+		1		+	++			1—5	+	−					living
von Verebély	+	+			9—3		+	++			1—6	++		C	+ −			dead
Jönsson, case no. 1					4		nodes	+		+	4—10		+	C		+		dead
Jönsson, case no. 2							+				14	−	−	−				living
Jönsson, case no. 3			+		o—2		+			+	o—4	+		C				dead
Jönsson, case no. 4		+			o—½		+	+		+				C				

Jönsson, case no. 4	+		+		o—½		+	+		+	5—2	+		C				dead
Jönsson, case no. 5			+		o—6		nodes			+	o—9		+			+		dead
Jönsson, case no. 6				+	4	+	+			+	4—5	+	+	C		+		dead
Jönsson, case no. 7			+		5		+	++		+	3—9	+	−	C				dead
Jönsson, case no. 8		+			o—6		+											
Jönsson, case no. 9			+		1		+			+	o—5							dead
Jönsson, case no. 10					o—1				+	+	1—6							living
Jönsson, case no. 11			+		o—2		+			+	1—10							living
Jönsson, case no. 12		+			1—6		+			+	1—6	+	+	C		+		dead
Jönsson, case no. 13							+			+	1—1							living
Berger, case no. 1					o—6		+					+						
Berger, case no. 2					o—6	+	+				o—5	+	+	C				dead
Berger, case no. 3					o—3		+				1—9	+		C				dead
Franseen *et al.*			+		1		+				1	+		C				dead
Albot *et al.*			+		o—11		+		+			+			+			
Fisher, case no. 1			+		o—3		+	+	+	+	2—4	++	−	+	++		+	dead
Fisher, case no. 2			+				+											living

* C = Clinical evidences only.

intercellular substance and often with an apparently definite cellular orientation. They undoubtedly warrant the name "endothelioma," as indefinitely restricted as this term is occasionally used (Ewing), but it is felt that such a tumor should not be included under the bimorphic synovial sarcoendotheliomas.

A number of cases remain unclassified. The cases of sarcoma reported by Krüger, case no. 2, 1903, and by Julliard and Descoeudres, 1904, might possibly be sarcoendotheliomas; in the latter case, which has been considered a synovioma by some (Jönsson), while the accompanying vague drawing suggests the papillary formation in a sarcoendothelioma, the author stated definitely that the "endothelium" lining the joint and covering the sarcomatous element was unchanged. The case reported by Marottoli (1936) contained one area that suggested a sarcoendothelioma, but the greater part of the tumor had a xanthomatous appearance. In Coley and Pierson's series of 15 cases, which included the 3 cases given by Adair (1935), individual case details were not given. A similar difficulty was met in Jönsson's large series (1938). In his general discussion of synovial tumors, Jönsson described sarcoendothelioma very carefully, but the inclusion of another tumor (case no. 82) in his series of synovialomas cast doubt upon the exact nature of the remainder. Personal communication (1939) definitely established these cases as sarcoendotheliomas. The case reported by Welch, Hampton and Mallory (1938) as synovioma, has Ewing's agreement as to its nature, but Mallory definitely stated that the tumor did not show a mixture of epithelial and spindle-cell elements. Bursell and Gellerstedt (1937) reported a possible sarcoendothelioma which they hesitated to classify definitely, and they pointed out the similarity of these tumors to the chronic hemorrhagic villous arthritis reported by Mandl. Finally, Pack (1939), in the legend to a picture, mentioned a synovioma without giving further details.

I was unable to obtain the original publications of Thomashoff (1896), Vinogradoff (1913), Barbacci (1915), Hohenthal (1934) and Bertini (1936). Bertini considered his tumor to be like that of Wegelin, but the microscopic features were not given in the only review that was obtainable.

Study of the Collected Cases

The features of synovial sarcoendothelioma can be analyzed from this series of 45 collected cases (Tables I and II).

Age and Sex

The age at onset of the first symptom varied from 13 to 70 years, with a median of 35 years. No tumors developed before puberty. From the time of puberty on, the tumor occurs at all ages and with nearly equal frequency (when the age distribution of the normal population is considered). Males and females are affected equally (21 : 22).

Site

The region of the knee joint is by far that most frequently involved, 14 tumors having this location (Text-Fig. 1). The remaining tumors were distributed almost equally in the wrist (3), hand (5), elbow (4), upper arm (2), shoulder (5), thigh (5), ankle and foot (4) and temporal region (1). Fourteen of the tumors arose from a joint capsule, 14 from a bursa, 7 from a tendon sheath; in 7 cases a choice between a tendon sheath or bursa could not be made.

Symptoms

The first symptom is noted in 35 cases. Local swelling was the initial clinical feature in 22 cases; in 11 the condition was heralded by pain. In only 2 cases was limitation of motion of the joint the symptom of onset. Of all the symptoms recorded before the primary operation, pain was noted 21 times, and limitation of motion 9. Seldom was a tumor tender on palpation, as was that of Knox, case no. 2 (1936).

Rate of Growth

Some of the primary tumors had a very slow rate of growth. In 14 instances operation was not performed for 3 or more years; in Fievez's case it was 10 years. Other authors (Fehr, Jönsson) have commented on a primary slowness of growth. This was not the rule in the entire series, for at least 14 were operated upon within the first year. The frequently long duration of symptoms before obtaining medical aid indicates an originally slow growth, and little early disability.

Gross Appearance of the Primary Tumor

The primary tumors varied from the size of a pea (Jönsson) to 20 cm. in diameter (Bonne and Collet). Their consistency was variable; in 16 tumors it was firm, in 11 it was soft, in a few instances the tumor was described as semi-fluctuant or pseudo-fluctuant before it was removed. While occasionally the tumor has been described as an intra-articular polypoid mass (Prym), it is usually a discrete mass located in the soft tissues, and when involving the joint synovia it is

Text-Figure 1. Approximate location of the reported cases of sarcoendothelioma.

customarily placed laterally to the articular surfaces. When examined *in situ* many seem to be well defined tumors, but they often give the impression of being fixed to underlying structures. An apparent capsule has been described in a number of instances. Of the 15 cases in which infiltration was mentioned, 8 had a visibly infiltrating margin. On several occasions the skin overlying the tumor has been ulcerated (Lejars and Rubens-Duval, Smith, case no. 3, Jönsson, case no. 5). The tumors most frequently had a white or gray-white internal color. The microscopic structure of the tumor suggests that small cystic spaces or clefts should often be seen in the cut surface of the tumor, and might be of aid in suspecting the nature of this tumor from the gross examination. Small cysts or clefts were seen by the naked eye in 11 instances: for only 2 was it recorded that cysts were not visible. In 7 of the 11 cases a clear fluid content of the cysts was observed.

Recorded experience indicates that usually the malignant nature of this tumor has not been suspected from naked-eye examination alone. While the gross appearance of sarcoendothelioma cannot be considered as characteristic, this tumor should be suspected when a tumor from an appropriate location has a cut surface that is gray-white and shows very small cysts or clefts that contain a clear, viscid fluid.

Roentgenographic study of the adjacent bony structures has seldom revealed any abnormality, being negative in at least 12 cases. Local atrophy of the nearby bone may occur as a secondary nutritional effect (Fievez, Fisher, case no. 1); direct bony invasion is reported in only 2 instances (Prym, Jönsson, case no 9).

Microscopic Appearance

By original selection each of these tumors had both a sarcomatous element and an epithelial-like tissue, a bilaterality of microscopic appearance that is the essential manifestation of sarcoendothelioma (Lejars and Rubens-Duval). The sarcomatous portion varies greatly, from a rather mature fibrillar tissue to an extremely cellular one in which few fibers are seen between the closely packed oval nuclei. Commonly both extremes are seen in the same tumor. The nuclei are usually only moderately irregular in size and mitotic figures are frequently observed (recorded in at least 18 cases). Occasionally there are areas of large polymorphous cells with large nuclei. There is some tendency to hyalinization (Sabrazès, 1932); and incidental features such as giant cells, xanthomatous cells and doubly-refractile fat droplets (Wegelin, 1928), polymorphonuclear leukocytes (Chenot and Tzanck) and mast cells (Lejars and Rubens-Duval) have been occasionally reported.

The epithelial-like tissue is usually seen lining gland-like spaces, or clefts or small cysts, less frequently as covering papillary projections, or forming solid cords or islands of cells. The cells have a round or moderately oval nucleus in which the chromatin is distributed in fine granules, and nucleoli are not prominent. Mitoses are often seen (noted in at least 11 cases). The cytoplasm is moderate in amount, clear, tends to be eosinophilic and occasionally contains fat droplets and small mucous vacuoles (Wegelin). When lining spaces the cells vary from cylindrical to flattened and are usually in a single cell layer but can be two to three cells thick or have the appearance of a syncytium (Chenot and Tzanck). This lining layer is not always complete. Mucus is always found in the contents of the glandlike tubes and small cysts when the mucicarmine stain is used. In regions where the epithelial-like formations are prominent, the scant connective tissue can appear mature, so that a hasty glance at the section is apt to give the impression

that one is dealing with an epithelial neoplasm until the cellular sarcomatous stroma is recognized. Areas in different tumors have been likened to carcinoma of the breast (Lejars and Rubens-Duval), carcinoma of the intestine and uterus (Chenot and Tzanck), mixed tumor (Schwamm), cylindroma (Smith), hypernephroma (Enderlen) and thyroid tumor metastasis (Wegelin). Brunner's case was first diagnosed an adamantinoma.

Epithelial-like and sarcomatous features are in varying proportions in the same and in different tumors. Since the original observation of Lejars and Rubens-Duval, it has been repeatedly noticed that in some areas one can find the sarcomatous element merging with the epithelial-like formation in such a gradual transition that it is impossible to fix a boundary between the two. These transitions form the strongest morphologic argument for the common ancestry of these dissimilar components.

Recurrence and Metastases

The malignant nature of this tumor is shown by the high rate of recurrence, which was 23 in 30 local excisions that had complete follow-up study. Re-recurrence was noted 4 times. Clinical evidence of spread to the regional lymph nodes was found in 10 instances, 6 of which were confirmed by microscopic examination. Metastasis to the lungs was reported in 22 cases on the basis of clinical study, of which only 3 were confirmed by postmortem examination. Instances of metastasis to the heart and jejunum (Wegelin) and to the spine, clavicle and soft parts (Jönsson) have been recorded.

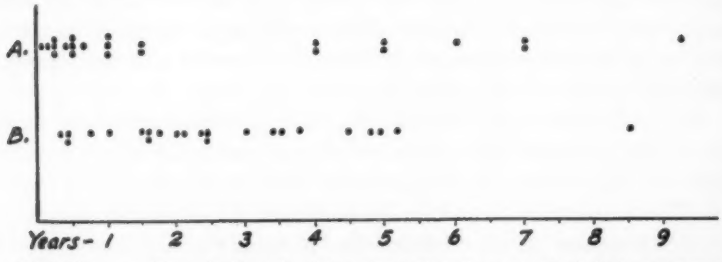
The recurrent tumors showed the same histologic structure as the original in five cases. In three cases the recurrences were pure sarcoma. The transition of the local recurrences from a sarcoendotheliomatous to a sarcomatous form is nicely shown by von Verebély's case in which the first recurrent tissue exhibited the epithelial-like elements in diminished number; the second showed them completely disappeared, the tumor being purely sarcomatous. In my case no. 1 both recurrences retained the structure of the original tumor. The lymph node metastases in five cases had the bimorphic appearance. In Zwahlen's case no. 2, metastases to the lung were found at autopsy, but no histologic description of them is given. Wegelin expressed the view that secondary growths tend to become more completely sarcomatous, and that the distant metastases contain only sarcomatous elements. On the basis of this single histologically examined case of Wegelin the tendency of pulmonary metastases to assume the sarcomatous form has been accepted by Gruber. My case no. 1 had pulmonary metastases that

showed both the epithelial-like and the sarcomatous portions, the former often in preponderance, proving that the pulmonary metastases of synovial sarcoendothelioma need not be exclusively sarcomatous; they can exhibit the same complex structure seen in the primary tumor.

Treatment

Every case was subjected to some type of surgical investigation or treatment. The majority (40) had a primary local excision. Amputation was the first operation in 2 cases. Radical reoperation, amputation, or both, was resorted to because of recurrent growth in 19 cases. Almost all authors commenting on treatment recommended early amputation as preferable (Sabrazès, Zwahlen, Knox).

X-ray therapy was used in 16 cases, usually as an adjuvant measure. Most authors have considered these tumors radioresistant (Fehr, Brunschwig). Jönsson holds the opposite viewpoint, that they are radiosensitive, and his large experience with synovial tumors gives his opinion considerable weight. The phenomenon of the transient disappearance of the shadows of the pulmonary metastases in my case no. 1 following intensive x-ray treatment suggests that these tumors may be temporarily radiosensitive. The term "radiosensitive" is a relative one, however, and no cure through the agency of roentgen therapy has been demonstrated.



Text-Figure 2. Row A gives the intervals of time from onset of first symptom to institution of surgical treatment; row B, intervals from first treatment until death. The mean of the latter intervals for this group of 23 cases is 2.7 years, standard deviation, 1.9.

No untreated patient, with the diagnosis first made by autopsy, has yet been reported, and no reported patient has developed metastases until after operation. These facts raise the question whether the tumor is activated into malignant growth by operative intervention. If this actually happens, there should be an interval of time between operation and death that is relatively constant. This point can be studied in the 23 cases in which sufficient data are given (Text-Fig. 2). The absence of untreated controls prevents any definite conclusion, but a tendency

to develop metastases after operation is certainly suggested. This tumor, when operated upon, should be given treatment appropriate for one of very malignant potentialities.

Prognosis

One is struck by the futility of most of the treatment so far recorded. Twenty-seven patients were known to be dead when they were reported; 25 due to the tumor. The other two were Prym's case of "marasmus," Jönsson's case no. 5 of pulmonary tuberculosis. Of the 7 patients known to be living, 6 had a duration of disease under 2½ years. Only Jönsson's patient, case no. 2, living 14 years after treatment, exceeded the customary "5-year cure" limit. It is possible that some benign forms of this condition do occur, as is suggested by Wegelin and Sabrazès. This is maintained by Black. One hesitates to make a dogmatic statement on this small series of cases, for as Jönsson points out this neoplasm is probably more common than the few reported cases would lead one to believe, but with the facts at hand one can give synovial sarcoendothelioma only a most pessimistic prognosis.

The Nature of Sarcoendothelioma

It was originally pointed out by Lejars and Rubens-Duval that the complex microscopic features of sarcoendothelioma could be explained by a consideration of the normal development and histologic structure of synovial membrane. The studies of His, Hagen-Torn, Hammar, Braun, Kroh and Lubosch brought liberation from the old idea of a distinct endothelium lining synovial membranes and developed our present concept of these structures as formed from the development of cavities in the preëxisting mesenchyme, which cavities were then lined by cells derived and only moderately differentiated from the specialized surrounding supportive connective tissue. The lining layer is termed "mesenchymal epithelium" (Jordan; Maximow and Bloom) in deference to its origin. Excellent detailed studies of the minute histologic features of synovial membrane have been given by Key, Sigurdson and Petersen. The differentiated surface cells of synovial membrane do not form a continuous lining layer, and despite their epithelial appearance, evidence of transition from the underlying connective tissue can be found. Cytologic details, like the fine chromatin structures in the nucleus and the relationship of cells to fibrils, indicate their connective tissue source. The growth behavior of the synovial cells is sufficiently individual, however, to lead Vaubel to consider them of definitely specialized morphology, under the name of synovioblasts.

In synovial sarcoendothelioma we have a malignant tumor that in

true neoplastic fashion carries a form of tissue growth to excess. The epithelial-like component can be looked upon as a malignant exaggeration in the differentiation of surface synovial cells. Evidences of its basic nature remain in the coincident sarcomatous proliferation of the supporting connective tissue and in the presence of transitions between these two elements. The occurrence of mucus in the glandlike spaces and cysts of the tumor, demonstrated by the mucicarmine stain, is the neoplastic counterpart of the mucin production studied by Cherry and Ghormley in synovia and by Vaubel in tissue culture.

The Name of the Tumor

Few tumors have been designated by more names than has this one. The original name of *endothéliome synovial* given by Lejars and Rubens-Duval in 1910 was consistent with the general concepts of that time. As related earlier, other appellations were applied to this tumor which were purely descriptive of its complex microscopic picture. The latest, conferred by Albot, Thiebaut, Banzet and Hervy in 1939, was *sarcomes fibroblastiques à type endo-épithélial*. Smith, recognizing the difficulty of using the term "endothelioma" when embryologic and histologic advances had made it inappropriate, and yet wishing to evade the use of any histogenetic name that might be based on knowledge still open to controversy, chose the term *synovioma*. This name was in a large measure satisfactory, as it evaded controversy and yet bespoke an identity. The weakness of such an "organ" name for a tumor is that it can be indiscriminately applied to all tumors from that location, and the 13 years that have passed since the name was first used have seen its exploitation in just this way. Wegelin's name of *sarco-endothelioma* has the advantages that it preserves the strict identity of the tumor and indicates its malignant nature, but the latter half of the name is no longer in accord with the more recent histogenetic concepts. The development of synovial membrane exclusively from mesodermal tissue is a generally accepted fact, consequently, *-mesothelioma* can be substituted for *-endothelioma*. The term, standing alone, would find confusion with the tumors (*e.g.*, of the pleura) that are now called mesothelioma. The prefix *sarco-* has the advantage of definitely indicating a malignant tumor of connective tissue origin. Considering these several features, I would suggest the use of the name *synovial sarco-mesothelioma* for the tumor discussed in this paper. This is done with great temerity, for the field is now overburdened with a multiplicity of names. It may be that subsequent investigation will show more features in common between this and other tumors arising from the synovial membranes, and thus increase the justification for the term synovioma,

or synovialoma, but in the present state of our knowledge I feel that this tumor is of such an individually complex structure that it merits its own individual name.

Summary

A bimorphic cellular constituency is considered to be the feature common to a small group of malignant tumors of the synovial membranes. This tumor has been given many names, including synovioma (Smith) and synovial sarcoendothelioma (Wegelin).

Two such tumors are here reported. One of them exhibits recurrent growth and pulmonary metastases which retain the bimorphic microscopic appearance.

From a review of the literature, 43 previously reported cases have been collected; the clinical and pathologic features of this series of tumors are analyzed.

The origin and nature of the tumor are discussed, and, as a compromise between usage and histogenesis, the name *synovial sarcomesothelioma* is proposed.

The salient features of the tumor are: that the tumor occurs with equal frequency at any age after puberty, it is found equally in both sexes and it springs from joint capsule, bursa and tendon sheath locations, most frequently in the region of the knee joint. Its initial clinical feature is usually swelling, but pain is the first symptom in one-third of the cases. The original rate of growth can be very slow. Gross examination of the tumor usually has not suggested its nature. Synovial sarcomesothelioma should be suspected if a gray-white tumor from an appropriate location contains small cysts or clefts. The primary tumor contains both epithelial-like and sarcomatous cellular features; these may be retained in metastases, or metastases may be purely sarcomatous. This tumor is malignant, recurs after local removal and metastases occur particularly in the lungs. There are no reports of such a malignant tumor completing its life history without surgical intervention.

BIBLIOGRAPHY

Adair, F. E. Discussion: synovial tumors. *Ann. Surg.*, 1935, 101, 810–812.

Albot, G.; Thiebaut, F.; Banzet, P., and Hervy, J. Sarcome fibroblastique et endo-épithélial de l'articulation du coude. *Bull. Assoc. franç. p. l'étude du cancer*, 1939, **28**, 589–602.

Barbacci, Ottone. I tumori. Casa Editrice, Francesco Vallardi, Milano, 1915. (Cited by Faccini.)

Berger, Louis. Synovial sarcomas in serous bursae and tendon sheaths. *Am. J. Cancer*, 1938, **34**, 501–539.

Bertini, G. Raro tumore primitivo post-traumatico della capsula articolare del ginocchio (endotelioma sinoviale). *Infortun. e Traumat. Lav.*, 1936, **2**, 159–172. (Reviewed in: *Zentralorgan. f. Chir.*, 1937, **81**, 319.)

Black, W. C. Synovioma of the hand. Report of a case. *Am. J. Cancer*, 1936, **28**, 481–484.

Bogetti, Hugo. Angioendotelioma de dedo. *Prensa méd. argent.*, 1937, **24**, 402–405.

Bonne, C., and Collet, A. M. Synoviomen. *Geneesk. tijdschr. v. Nederl.-Indië*, 1935, **75**, 1384–1390.

Braun, Heinrich. Untersuchungen über den Bau der Synovialmembranen und Gelenkknorpel, sowie über die Resorption flüssiger und fester Körpel aus den Gelenkhöhlen. *Deutsche Ztschr. f. Chir.*, 1894, **39**, 35–86.

Brunner, R. Zur Frage der ortsungewöhnlichen Adamantinome und Speicheldrüsenmischgeschwülste und ihrer Beziehungen zur den Synoviomen. Inaugural dissertation, Zürich, 1936. (Cited by Fehr.)

Brunschwig, Alexander. Tumors of the synovia, tendons, and joint capsules of the hands and feet. *Surgery*, 1939, **5**, 101–111.

Burckhardt, Hans. Ein primäres Sarkom der Kniegelenkskapsel. *Deutsche Ztschr. f. Chir.*, 1909, **101**, 467–473.

Bursell, S., and Gellerstedt, N. Zur Kenntnis der primären, bösartigen Skelettgeschwülste von epitheliomorphem Typus.—Zugleich einiges zur Endotheliomfrage. *Upsala läkaref. förh.*, 1937, **43**, 91–100.

Chenot, and Tzanck. Endothéliome synovial du pied. *Bull. et mém. Soc. anat. de Paris*, 1912, s.6, **14**, 293–296.

Cherry, J. H., and Ghormley, R. K. A histopathological study of the synovial membrane with mucicarmine staining. *J. Bone & Joint Surg.*, 1938, **20**, 48–56.

Chiari, H. Die Geschwülste der Gelenke. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1934, **9**, pt. 2, 75–106.

Coley, B. L., and Pierson, J. C. Synovioma. Report of fifteen cases with review of literature. *Surgery*, 1937, **1**, 113–124.

Diez, Julio. El endotelioma sinovial articular. *Prensa méd. argent.*, 1931, **18**, 487–500.

Enderlen. Über Geschwülste der Kniegelenkkapsel. *Med. Klinik*, 1920, **16**, 721–724.

Ewing, James. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1940, ed. 4, pp. 335–385.

Faccini, U. Un caso di peritelioma sarcomatoso della capsula articolare del ginocchio destro. *Arch. ital. di chir.*, 1923, **7**, 481–501.

Fehr, A. Zur Klinik und Pathologie der Synoviome. *Beitr. z. klin. Chir.*, 1937, **165**, 88–104.

Fievez. Endothéliome de l'articulation du cou-de-pied. *Bull. et mém. Soc. nat. de chir.*, 1935, **61**, 1034–1037.

Franseen, C. C.; Simmons, C. C., and Mallory, T. B. Synovioma. Massachusetts General Hospital, case no. 25312. *New England J. Med.*, 1939, **221**, 196–198.

Geschickter, C. F., and Lewis, Dean. Tumors of tendon sheaths, joints, and bursae. *Am. J. Cancer*, 1934, **22**, 96–126.

Giraud, P.; Salmon, M., and Paillas, J. E. Tumeur à myéloplaxes de la synoviale du genou. *Ann. d'anat. path.*, 1939, **16**, 548–555.

Gruber, G. B. In: Kaufmann, Eduard. Lehrbuch der speziellen pathologischen Anatomie. Walter de Gruyter Co., Berlin, 1938, ed. 10, **2**, pt. 1, p. 1316.

Hagen-Torn, O. Entwickelung und Bau der Synovialmembranen. *Arch. f. mikr. Anat.*, 1882, **21**, 591–663.

Hammar, J. A. Ueber den feineren Bau der Gelenke. *Arch. f. mikr. Anat.*, 1894, **43**, 266–326.

Harbitz, Francis. Tumors of tendon sheaths, joint capsules and multiple xanthoma. *Arch. Path.*, 1927, **4**, 507–527.

His, Wilhelm. Die Häute und Höhlen des Körpers. Basel, 1865.

Hodgson, F. G., and Bishop, E. L. Malignant synovioma of the knee joint. *J. Bone & Joint Surg.*, 1935, **17**, 184–188.

Hohenthal, T. Ett fall av primart knaledkapselsarkom. *Finska läk.-sällsk. handl.*, 1934, **76**, 458–476. (Cited by Knox and by Jönsson.)

Janik, Alfred. Tumors of tendon sheaths. *Ann. Surg.*, 1927, **85**, 897–911.

Jönsson, G. Malignant tumors of the skeletal muscles, fasciae, joint capsules, tendon sheaths and serous bursae. *Acta radiol.*, 1938, suppl. **36**, 1–304.

Jönsson, G. Personal communication. Stockholm, May 13, 1939.

Jordan, H. E. A Textbook of Histology. D. Appleton & Co., New York, 1930, ed. 5.

Julliard, Charles, and Descoeudres, Francis. Sarcome primitif de la synoviale du genou. *Arch. internat. de chir.*, 1904, **1**, 539–571.

Key, J. A. The Synovial Membrane of Joints and Bursae. In: Cowdry, E. V. Special Cytology. P. B. Hoeber, Inc., New York, 1928, **2**, 737–756.

Knox, L. C. Synovioma: a report of three cases. *Arch. Path.*, 1934, **18**, 926–928.

Knox, L. C. Synovial sarcoma. *Am. J. Cancer*, 1936, **28**, 461–480.

Kroh, Fritz. Studien über den Bau der Synovialmembran und die Resorption des Gelenkinhaltes unter dem Einflusse variabler-mechanischer Momente. *Deutsche Ztschr. f. Chir.*, 1908, **94**, 215–240.

Krüger, J. H. Zur Anatomie und Klinik der primären Sarkome im Bereich der Kniegelenkskapsel. Inaugural dissertation. Bruno Georgi, Leipzig, 1903.

Lejars, and Rubens-Duval. Les sarcomes primitifs des synoviales articulaires. *Rev. de chir., Paris*, 1910, **41**, 751–783.

Lubosch, Wilhelm. Bau und Entstehung der Wirbeltiergelenke; eine morphologische und histogenetische Untersuchung. G. Fischer, Jena, 1910.

Mandl, Felix. Chronische Arthritis villosa haemorrhagica des Kniegelenkes. *Zentralbl. f. Chir.*, 1928, **55**, 597–601.

Marottoli, O. R. Sinovioma del cuello del pie. *An. de cir.*, 1936, **2**, 391–400.

Maximow, A. A., and Bloom, William. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930, pp. 191–194.

Pack, G. T. Symposium: tumors of the hands and feet. Introduction. *Surgery*, 1939, **5**, 1–26.

Petersen, H. In: v. Möllendorff, Wilhelm. Handbuch der mikroskopischen Anatomie des Menschen. Julius Springer, Berlin, 1930, **2**, pt. 2, 659–672.

Prym, P. Synoviales Sarkoendotheliom des Kniegelenks. *Virchows Arch. f. path. Anat.*, 1931, **279**, 71–78.

Razemon, P., and Bizard, G. Les tumeurs primitives des articulations. *Rev. de chir., Paris*, 1931, **69**, 229–266.

Sabrazès, J., and de Grailly, R. Synovialome. *Compt. rend. Soc. de biol.*, 1931, **106**, 1158–1161.

Sabrazès, J.; Loubat, E.; de Grailly, R., and Magendie, J. Les tumeurs articulaires. Synovialomes malins polykystiques. *Gaz. hebd. d. sc. méd. de Bordeaux*, 1932, **53**, 481–490.

Sabrazès, J.; Loubat, E.; de Grailly, R., and Magendie, J. Synovialosarcomes. *Gaz. hebd. d. sc. méd. de Bordeaux*, 1934, **55**, 754–762.

Schwamm, Max. Zur Frage der Geschwülste in Schleimbeuteln. *Zentralbl. f. Chir.*, 1930, **57**, 2478–2484.

Sigurdson, L. A. The structure and function of articular synovial membranes. *J. Bone & Joint Surg.*, 1930, **12**, 603–639.

Smith, L. W. Synoviomata. *Am. J. Path.*, 1927, **3**, 355–364.

Spencer, W. G. Discussion of Turner and Lockwood's cases of sarcoma of the synovial membrane. *Lancet*, 1902, **1**, 1398.

Stewart, F. W. Discussion of W. B. Coley's presentation at the meeting of the New York Surgical Society, April 25, 1934. *Ann. Surg.*, 1935, **101**, 806.

Stüer, Joseph. Eine ungewöhnliche Geschwulst der Ellbogengelenksgegend. Inaugural dissertation, Würzburg, 1893.

Tavernier. Sarcome de la synoviale du genou. *Lyon chir.*, 1930, **27**, 522–524.

Thomashoff, Fritz. Beitrag zur Casuistik der sarcomatösen Geschwülste des Kniegelenks. Inaugural dissertation, Würzburg, 1896. (Cited by Sabrazès *et al.*)

Vaubel, Ernst. The form and function of the synovial cells in tissue cultures. I. Morphology of the cells under varying conditions. *J. Exper. Med.*, 1933, **58**, 63–83.

Vaubel, Ernst. The form and function of synovial cells in tissue cultures. II. The production of mucin. *J. Exper. Med.*, 1933, **58**, 85–95.

Vinogradoff. Un cas de sarcome de l'articulation du genou. *Chirourgia*, 1913, **34**, 345. (Cited by Razemon and Bizard.)

von Rosen, Sophus. Ein Fall von synovialem Sarkoendotheliom des Kniegelenks. *Acta orthop. Scandinav.*, 1937, **8**, 191–207.

von Verebély, T. Beiträge zur Pathohistologie der synovialen Erkrankungen. *Schweiz. med. Wchnschr.*, 1938, **19**, 458–461.

Wagner, L. C. Intra-articular endothelial tumors arising from synovial membrane. *Ann. Surg.*, 1930, **92**, 421–424.

Wagner, L. C. Discussion: synovial tumors. *Ann. Surg.*, 1935, **101**, 809–810.

Wegelin, C. Ueber falsche und echte Tumoren der Kniegelenkskapsel. *Schweiz. med. Wchnschr.*, 1928, **9**, 722–726.

Wegelin, C. Pathologisch-anatomische Demonstration [Fall 6]. *Schweiz. med. Wchnschr.*, 1932, **13**, 241–242.

Welch, C. A.; Hampton, A. O., and Mallory, T. B. Synovioma. Massachusetts General Hospital, case no. 24321. *New England J. Med.*, 1938, **219**, 204–206.

Zwahlen, Paul. Sur les synoviomes des gaines tendineuses et des bourses séreuses. *Bull. Assoc. franç. p. l'étude du cancer*, 1935, **24**, 682–707.

SUPPLEMENTARY BIBLIOGRAPHY

Cases not Classifiable as Synovial Sarcoendothelioma

Adrian, C. Ueber die von Schleimbeuteln ausgehenden Neubildungen. *Beitr. z. klin. Chir.*, 1903, **38**, 459–485. (Fibrosarcoma, 2 cases.)

Agrifoglio, M. Contributo allo studio dei tumori delle guaine tendinee. *Ann. ital. di chir.*, 1928, **7**, 1220–1243. (Xanthoma.)

Annandale. Internal derangements of the knee-joint. Read before the Medico-Chirurgical Society of Edinburgh, 1886. *Lancet*, 1894, **2**, 54. (Cited by Turner.) (Sarcoma.)

Bánfalvi, Tivadar. Térdizületi daganat ritka esete. *Orvosi hetil.*, 1935, **79**, 1293–1294. (Xanthoma.)

Blumenthal, Max. Primäres, diffuses Sarkom der Kniegelenkkapsel. *Deutsche med. Wchnschr.*, 1912, **38**, 2305–2307. (Xanthoma.)

Coley, W. B. Malignant tumor of synovial membrane of knee-joint. *Ann. Surg.*, 1935, **101**, 805–809. (Fibrosarcoma.)

Dowd, C. N. Villous arthritis of the knee (sarcoma). *Ann. Surg.*, 1912, **56**, 363–365.

Faulkner, D. M. Primary synovial membrane tumors of joints. *Surg., Gynec. & Obst.*, 1931, **53**, 189–195. (Giant cell xanthoma.)

Fedeli, Fedele. Contributo alla conoscenza dei tumori delle articolazioni (Mixocondrosarcoma della capsula articolare del ginocchio). *Chir. d. org. di movimento*, 1925–26, **10**, 209–224. (Myxochondrosarcoma.)

Garrè, C. Diffuses Sarkom der Kniegelenkskapsel. *Beitr. z. klin. Chir.*, 1891, **7**, 232–237. (Sarcoma.)

Gömöri, G. Three uncommon tumors. *Am. J. Surg.*, 1936, n. s. **33**, 150–156. (Case no. 3, xanthoma.)

Hannemüller. Ueber primäre Sarkome der Gelenkkapsel. *Beitr. z. klin. Chir.*, 1909, **63**, 307–314. (Sarcoma.)

Harbitz, Francis. Primaere svulster i ledkapsler. Diffuse sarkomer i ledkapselen under billedet av en tuberkulose. *Norsk mag. f. laegevidensk.*, 1927, **88**, 369–375. (Xanthoma.)

Hardie. A case of sarcomatous degeneration of the synovial membrane of the knee-joint [case of S. C. Salter]. *Lancet*, 1894, **1**, 1619–1620. (Sarcoma.)

Hartman, F. W. Synovial membrane tumors of joints. *Surg., Gynec. & Obst.*, 1922, **34**, 161–167. (Giant cell xanthoma.)

Hutchinson, Jonathan, Jr. Discussion of case reported by Howard Marsh. *Lancet*, 1898, **2**, 1330–1331. (Sarcoma.)

Iovino, Ferdinando. Contributo alla conoscenza dei tumori primitivi delle articolazioni. Su di un mixoma sarcomatoso del ginocchio. *Pathologica*, 1934, **26**, 98–104. (Myxosarcoma.)

La Ferla, Luigi, and Demelas, Giuseppe. Sinovialoma (istiocitoma) del ginocchio. *Chir. d. org. di movimento*, 1934–35, **19**, 239–252. (Giant cell xanthoma.)

Langemak, O. Zur Kenntniss der Chondrome und anderer seltener Geschwülste der Gelenke. *Arch. f. klin. Chir.*, 1904, **72**, 55–63. (Xanthoma, 2 cases.)

Lockwood, C. B. Sarcoma of the synovial membrane of the knee-joint. *Lancet*, 1902, **1**, 1398. (Sarcoma.)

Marsh, Howard. Primary sarcoma of the synovial membrane of the knee-joint. *Lancet*, 1898, **2**, 1330–1331. (Sarcoma.)

Marsh, Howard. Discussion of cases of sarcoma of synovial membrane presented by Turner and Lockwood. *Lancet*, 1902, **1**, 1398. (Sarcoma.)

Martina, A. Ein Beitrag zur Kenntnis der Schleimbeuteltumoren. *Deutsche Ztschr. f. Chir.*, 1906, **83**, 317–323. (Myxosarcoma.)

Moser, Ernst. Primäres Sarkom der Fussgelenkkapsel. Exstirpation. Dauerheilung. *Deutsche Ztschr. f. Chir.*, 1909, **98**, 306–310. (Xanthoma.)

Mouchet, Alain; Duperrat, and Vincent, Jean. Tumeur de la synoviale du genou. *Ann. d'anat. path.*, 1937, **14**, 252–255. (Xanthoma.)

Ranke, H. R. Ueber Geschwülste der Schleimbeutel. *Arch. f. klin. Chir.*, 1886, **33**, 406–426. (Myxoma.)

Salter, S. C. Discussion of presentation by Mr. Hardie, with case report. *Lancet*, 1894, **1**, 1620. (Sarcoma.)

Savariaud. Tumeur maligne du genou—Dégénérescence myxomateuse de la synoviale. *Bull. et mém. Soc. anat. de Paris*, 1905, s.6, **7**, 890–893. (Myxosarcoma.)

Simeoni, C. Rara sindrome da neoplasma intravelico (mixo-endotelioma). *Riv. di chir.*, 1937, **3**, 140–143. (Myxoendothelioma.)

Simon, Gustav. Exstirpation einer sehr grossen, mit dickem Stiele angewachsenen Kniegelenkmaus mit glücklichem Erfolge. *Arch. f. klin. Chir.*, 1865, **6**, 573–576. (Xanthoma.)

Tourneux, J.-P. Les sarcomes des gaines tendineuses. *Rev. de chir., Paris*, 1913, **47**, 817–854. (Fibrosarcoma.)

Turner, A. L. Primary sarcoma of the synovial membrane. *Lancet*, 1894, **2**, 54. (Sarcoma.)

Turner, G. R. Primary sarcoma of the synovial membrane of the ankle-joint. *Lancet*, 1902, **1**, 1398. (Fibrochondrosarcoma.)

van Luijt, C. Een geval van primair sarcoom van de knie. *Nederl. tijdschr. v. geneesk.*, 1925, **69**, pt. 2A, 356–358. (Fibrosarcoma.)

van Rijssel, E. C. Over sarcoma van de gewrichtskapsel. *Nederl. tijdschr. v. geneesk.*, 1917, **61**, pt. 2, 1143–1148. (Fibrosarcoma.)

v. Ruediger-Rydygier, A. R. Zur Diagnose und Therapie des primären Sarkoms der Kniegelenkkapsel. *Deutsche Ztschr. f. Chir.*, 1906, **82**, 211–225. (Xanthoma, case no. 1; fibrosarcoma, case no. 2; small round cell sarcoma, case 3.)

Villard, Pierre–V.–J. Contribution à l'étude des sarcomes primitifs de la synoviale du genou. Thèse, Paris, 1929. (Xanthoma.)

Weir, R. F. On fatty and sarcomatous tumors of the knee-joint. *Med. Rec., N. Y.*, 1886, **29**, 725–727. (Abstracted in: *Zentralbl. f. Chir.*, 1886, **13**, 799–800.) (Xanthoma, fibrosarcoma.)

Züllig, J. Tumoren der Kniegelenkkapsel. *Cor.–Bl. f. schweiz. Aerzte*, 1917, **47**, 1368–1381. (Xanthoma.)

DESCRIPTION OF PLATES

PLATE 86

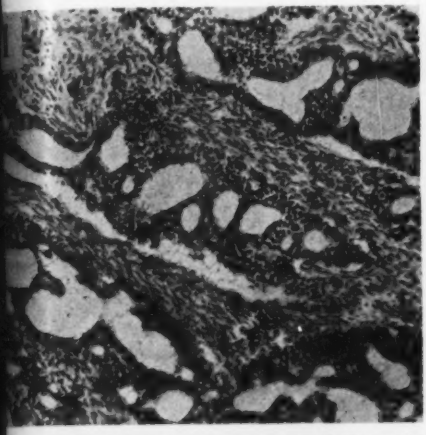
FIG. 1. Case no. 1. Epithelial-like formations of original tumor. × 90.

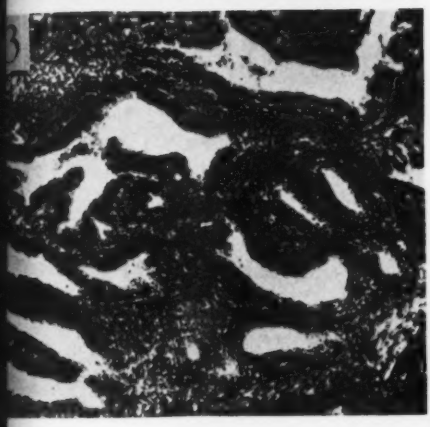
FIG. 2. Case no. 1. First recurrent tumor mass, nearly natural size.

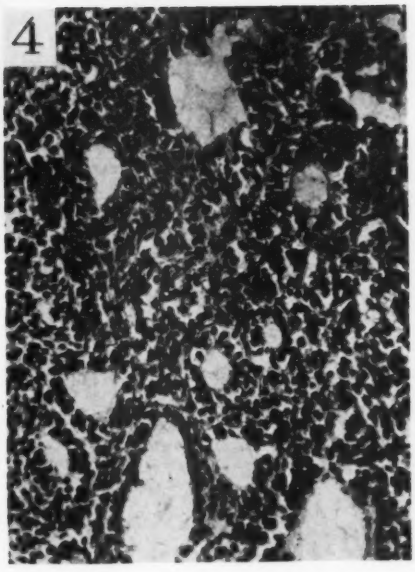
FIG. 3. Case no. 1. Section of first recurrence, showing spaces, papillary formation and lining layer. × 90.

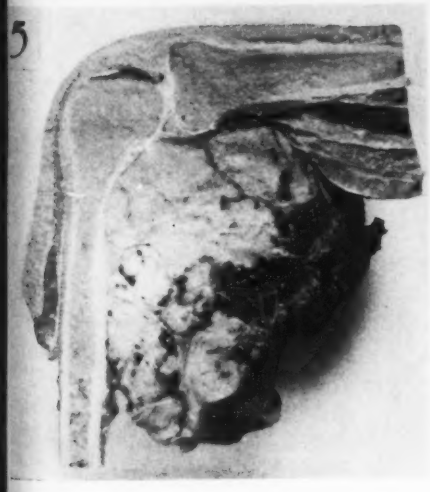
FIG. 4. Case no. 1. The space-lining cell layer occasionally merges with the stroma. × 325.

FIG. 5. Case no. 1. Longitudinal section through the amputated leg showing the second recurrent tumor mass. Greatly reduced.









Fisher

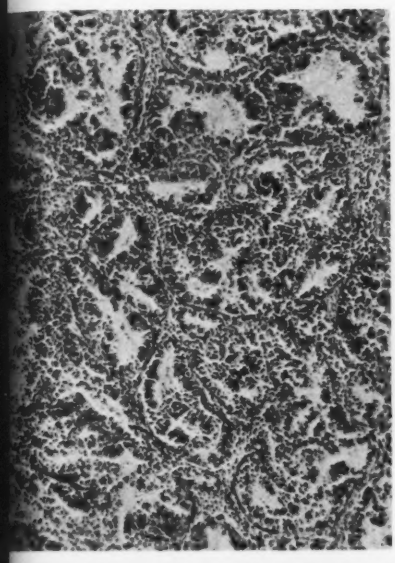
Synovial Sarcomesothelioma

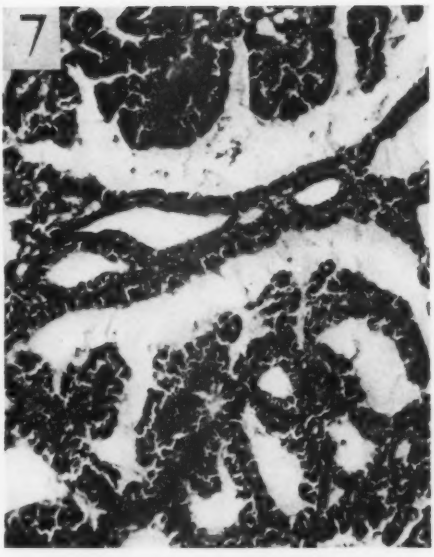
PLATE 87

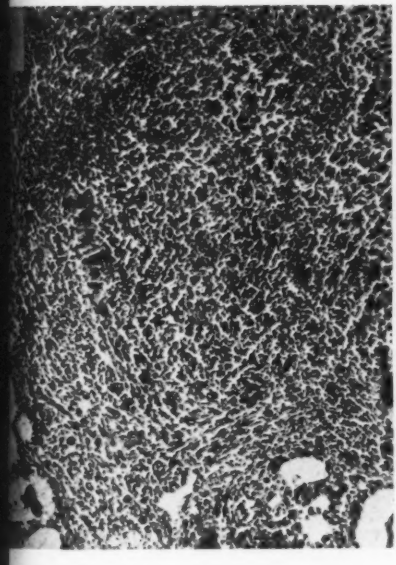
FIGS. 6 and 7. Case no. 1. Areas of the pulmonary metastases, showing adenomatous forms. × 100.

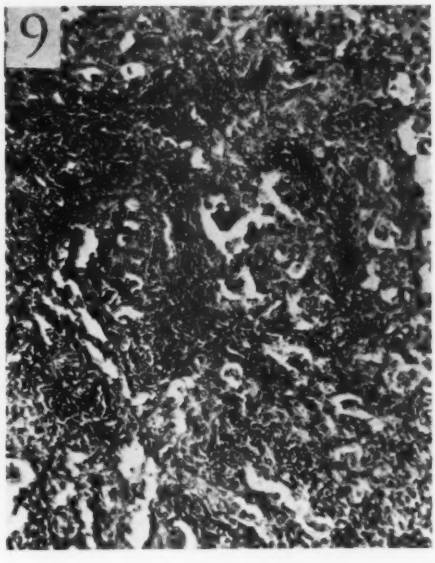
FIG. 8. Case no. 1. Sarcomatous area in metastasis to lung. × 100.

FIG. 9. Case no. 2. The cells lining the spaces, while differentiated from the stroma, have no definite basement membrane. × 100.









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